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REVIEW

Cancer stem cells: a reality, a myth, a fuzzy concept or a misnomer? An analysis

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The concept of cancer stem cells (CSC) embodies two aspects: the stem cell as the initial target of the oncogenic process and the existence of two populations of cells in cancers: the CSC and derived cells. The second is discussed in this review. CSC are defined as cells having three properties: a selectively endowed tumorigenic capacity, an ability to recreate the full repertoire of cancer cells of the parent tumor and the expression of a distinctive repertoire of surface biomarkers. In operational terms, the CSC are among all cancer cells those able to initiate a xenotransplant. Other explicit or implicit assumptions exist, including the concept of CSC as a single unique infrequent population of cells. To avoid such assumptions, we propose to use the operational term tumor-propagating cells (TPC); indeed, the cells that initiate transplants did not initiate the cancer. The experimental evidence supporting the explicit definition is analyzed. Cancers indeed contain a fraction of cells mainly responsible for the tumor development. However, there is evidence that these cells do not represent one homogenous population. Moreover, there is no evidence that the derived cells result from an asymmetric, qualitative and irreversible process. A more general model is proposed of which the CSC model could be one extreme case. We propose that the TPC are multiple evolutionary selected cancer cells with the most competitive properties [maintained by (epi-)genetic mechanisms], at least partially reversible, quantitative rather than qualitative and resulting from a stochastic rather than deterministic process.

Introduction

The superb demonstration of the role of stem cells in embryogenesis, and in the renewal of adult tissues such as blood, has quite naturally led to the concept that a similar model may apply to cancer (1–3). Many articles have therefore investigated and discussed the hypothesis of stem cells of cancers. These cells have been called cancer stem cells (CSC), tumor initiating cells (TIC) and stemloids. We believe that the operational term tumor-propagating cells (TPC) are more suitable (see Appendix for terminology) (4,5). However, as CSC is the most used term, we shall use it in the first part of this review.

Although still debated (6,7), the validity of the CSC concept is more and more taken as granted, sometimes as an undiscussed dogma. In many articles, all findings are interpreted within the framework of the model with no consideration of alternative explanations (8). Moreover, the fact that major commercial interests are involved does not help. The field remains, however, open not only with regard to the validity in all cases of the model but also with regard to the definition of CSC with its explicit criteria, their correlates and implicit assumptions. The purpose of this review is to analyze these as well as their experimental basis.

Abbreviations: CSC, cancer stem cells; ESC, embryonic stem cells; SSC, somatic stem cells; TIC, tumor-initiating cells; TPC, tumor-propagating cells.

The concept embodies two subconcepts: the somatic stem cells (SSC) as the initial target of carcinogenesis and the existence in cancer tissue of one single distinct population of CSC.

The first fundamental question whether cancer cells originate from SSC is not considered extensively here, although many authors clearly link the two concepts (9,10). The evidence in favor and against this concept is briefly discussed in supplementary Information 1 (available at *Carcinogenesis* Online) (11–15). The probable SSC origin of some cancers is largely independent of the concept of CSC that we shall discuss.

General aspects of somatic and embryonic stem cells

As the concept of CSC derives from the general model of stem cells, it is important to remind the definition of the latter (Table I).

There are at least two general types of stem cells: the embryonic stem cells (ESC) and the SSC. All are characterized by: (i) self-renewal and immortality (i.e. extended life span or number of possible divisions); (ii) asymmetric divisions with irreversible sequential generation of a hierarchy of more differentiated descendants, with a limited life span, steadily reproducing the cell heterogeneity of tissues; this does not exclude some symmetrical divisions generating two stem cells and (iii) homeostatic control (16). The differentiation is a deterministic process. The fertilized oocyte can be considered as the initiator of all stem cells, the primary stem cell (16). Stem cells of both types give rise to hierarchies of stem cells and derived pluripotential cells with a more restricted range of possible outcomes at each stage.

ESC and SSC differ in several respects. While ESC are able to generate the three germ layers and ultimately all differentiated cells, SSC have a much more restricted potential (17). Both types use different pathways for their maintenance (18). ESC, which express telomerase, are immortal, whereas the exhaustion of the replicative capacity of adult stem cells may contribute to aging (17). While ESC reproduce rapidly, the division rate of most SSC is believed to be low although this is becoming controversial (19,20). To maintain their undifferentiated open state, SSC require a very sophisticated but specific microenvironment with which they interact: the niche (16). The ESC only need to be protected from differentiating agents (21). The characteristic and phenotype directing transcription factors of ESC (OCT4, SOX2, NANOG, TCF3) are not expressed in SSC, each type of which expresses transcription factors specific or not of the stem cell status or of the cell identity—SSC may exist in many tissues (22) (supplementary Information 2 is available at *Carcinogenesis* Online).

ESC give rise to a hierarchy of progressively fate-restricted pluripotent cells. SSC differ much, depending on the organ in which they reside, but each type can also give rise to a sequential panoply of less and less pluripotent, then terminally differentiated cells (23). There is good evidence that SSC are resistant to apoptosis, X rays and chemotherapy.

The paradigmatic irreversibility of stem cell differentiation should be qualified in view of the successful transformation of fully differentiated adult cells in ESC analogs, by the action of a few ESC transcription factors: OCT4, SOX2, NANOG and LIN28 (24,25).

There is therefore no unique model of stem cell or even of SSC.

The concept of CSC

Authors have different operational definitions of CSC. Nevertheless, general reviews have defined them by three properties (26). In the order proposed, the unique population of CSC (i) expresses a distinctive repertoire of surface markers that allow its reproducible and

Table I. Proposed characteristics of CSC and their derivatives as compared with the properties of normal stem cells

CSC properties			Normal stem cell properties
Minimal literature definition	Consequent correlates	Implicit questionable assumptions	
Selectively endowed. Tumorigenic capacity.	Self-renewal. Immortality. Only CSC cause successful grafts.	Hallmarks of cancer cells: - Self-sufficiency in growth signals. - Insensitivity to anti growth signals. - Resistance to apoptosis.	Self-renewal. Immortality. Requirement for GF. Necessary niche. Resistance to apoptosis?
Hierarchical. Sustains the growth of heterogenous cancer. Tissue recreating the full repertoire of cancer cells.	CSC only a fraction of tumor.	Characteristics of whole tumor, e.g. gene expression signatures, apply mainly to the majority of derived cells.	Consistent minority, subpopulation of stem cell.
	CSC undifferentiated.		Undifferentiated.
	Differentiation of derived lineages.	Asymmetric divisions? Deterministic? Qualitative? Irreversible?	Differentiation with asymmetric division. Deterministic. Qualitative. Irreversible.
	Limited life span of derived lineages.		Limited life span of differentiated lineages.
Expression of distinctive repertoire of characteristics including surface markers and functional characteristics.	Functional characteristics: growth in spheres, in transplants, dye exclusion.	Markers well defined? Permanent? Intrinsic role?	Repertoires of markers.
	Resistance to X ray and chemotherapy.		Resistance to X rays, chemotherapy.
—	—	?	Homeostatic control.
—	—	?	Division rate: - High for ESC - Low for SSC

The characteristics of CSC are divided in those strictly and explicitly described in the definition, explicit correlates of the definition and frequent implicit assumptions.

differential purification; (ii) is selectively endowed of tumorigenic capacity as opposed to all other subsets and (iii) sustains the growth of heterogenous cancer tissue, recreating the full repertoire of cancer cell populations observed in the parent tumor (hallmarks: self-renewal and differentiation). These are very restricted criteria but we shall see that each of them has correlates and implies non-explicit assumptions. However, different researchers use different criteria and definitions and therefore distinguish cell properties that may differ in part with those defined hereinabove (27). Let us first explicit this minimal definition.

The expression of a distinctive repertoire of surface markers is an operational prerequisite for the isolation of the CSC population. However, it should be noted that the markers proposed are different for the different types of cancers and different from those of SSC. Their potential biological role is little considered and it is implicitly assumed that they permanently mark the same cells.

The selective endowment of a tumorigenic capacity implies that cancers contain at least two populations of cells, one being the CSC, which self-renew and are immortal, and the other being the derived population, which has a limited life span and therefore should be considered as an almost innocuous by-product. Many researchers, using the cell sorting vocabulary, call the putative CSC, the side population (27,28) which for an initiating group is counterintuitive. This criterion overlaps the third one, whereby the CSC population sustains the growth of heterogenous cancer tissue, recreating the full repertoire of cancer cell population i.e. a hierarchy of differentiated derived lineages.

A clinically interesting correlate of the CSC concept is that CSC would be resistant to chemotherapy and radiation, thus being responsible for recurrence of cancer after such therapies (29–33).

As seen from Table I, there are some clearly defined common points between the CSC and the SSC concept: absence or reversal of differentiation, self-renewal and immortality, the existence of biomarkers and generation of a hierarchy of derived lineages of finite life span. It has been proposed that both SSC and CSC share the property of resistance to hypoxia (34). Some characteristics of SSC, often implicitly assumed, perhaps because of the terminology, are debatable for CSC: the niche, asymmetry of division, deterministic process and qualitative irreversible transition from the CSC to the derived cells. It has been hypothesized that the derived cell population could constitute a niche for CSC (35) and that other cells would constitute a 'niche' for metastases (36). Homeostatic control of SSC population and selection of this process by long-term evolution clearly do not apply to CSC.

We shall now examine more in detail the experimental evidence supporting the validity of these various criteria and assumptions for various cancers that derive partly from the terminology used (CSC) and are often implicit in the reasoning and in data interpretation.

Evidence on the applicability of CSC characterization criteria to various cancers

First criterion/property: selectively endowed tumorigenic capacity of the CSC

This property implies the existence of a distinct fraction of the population of cancer cells having the properties of self-renewal, immortality and potential to cause tumors and of another heterogenous fraction of cells (the derived population) deprived of these properties i.e. only able to perform a limited number of divisions (Table II). This definition is a direct transposition of the SSC property if we replace tissue generation by the potential to cause tumors. An implicit assumption is that the two populations are qualitatively different (37) and do not represent both sides of a continuum.

Table II. Properties of somatic stem cells versus cancer stem cells and their derivatives

	SSC	TPC–CSC
I. Stem cells		
Asymmetry in division	+	Unknown
Difference with derived cells	Qualitative.	Quantitative?
Process of derived cells generation	Deterministic. Physiologically irreversible (but possible with ESC factors)	Stochastic? Reversible in part?
Mechanism of differentiation	Epigenetic only?	Functional, epigenetic and genetic?
Homeostatic control	+	No
Necessity of niche	+	Unknown
Tissue specific	+	+?
Division rate	Low	High
II. Derived cells		
Hierarchy	+	+?
	Organized.	Disorganized.
	Physiologically relevant.	Byproduct of disorganization. Waste with spillovers from CSC.
	Differentiation as default state.	Parody of differentiation. Differentiation, cell death, senescence as default states.
	Very proliferative in intermediate stage.	Poorly proliferative?
	Mechanism selected by evolution.	No

The hypothesis that such a population of cells would be single, unique and homogenous looks hardly possible as such cells even if they exist initially probably evolve and acquire different genetic or epigenetic properties leading to heterogeneity. This is suggested by several observations. CSC in acute myelogenous leukemia vary in self-renewal potency and quiescence status (38). Preleukemic stem cells are different from their descendent leukemic CSC in genotype, phenotype and frequency (5). Chronic myelogenous leukemia in blast crisis has a different population of self-renewing CSC than in the chronic phase (39).

The operational basis of this concept is the fact that a minimal number of cells (from 10^6 to 10^9 cells) must be injected in immunocompromised mice to produce a tumor. This would reflect the fact that only a fraction of these injected cells is able to generate a tumor: the CSC. However, alternative explanations are that statistically, and by chance, only a fraction of the injected cells succeed to land and to develop at a suitable site or that some cells are better suited for the new microenvironment, etc. It must be emphasized that, whatever the criterion used to define them, the reported fractions of CSC in the entire population vary by several orders of magnitude (from 0.1 to 30%) depending on the type of cancer and the study (40–42). The concept of metastases stem cells concerns among the CSC those that are able to metastasize (26,30,36,43).

The CSC hypothesis is reinforced when it is shown that by previous purification of CSC, e.g. using a biomarker, or by serial transplantation, the tumor yield is greatly increased. This certainly suggests that a fraction of the tumor cells have a superior ability to generate a tumor. In the case of human acute myeloid leukemia cells, the exclusive property of the minority of CD34+ CD38− cells to transfer leukemias to recipient NOD–SCID mice supports the conclusion (3,44). Similarly, the fact that single cell cloning of colon cancer cells with CSC properties (dye exclusion, expansion in spheroid cultures, CD133 and CD24 biomarkers) (35) or a rat mammary cell line (SLA7) selected for such properties can reproduce tumors *in vivo* and pass on their properties (45) in sequential propagations to some of their descendants is a strong argument in favor of a permanent property.

Most of the injection experiments concern xenotransplants of human cancer cells in immunocompromised mice, a species difference that does not favor implantation. This is influenced by the presence of residual immune effector cells in the recipient, the site of transplantation, the suitability of microenvironment, etc (43,46). As pointed out by Blagosklonny (47), millions of sperm cells are needed to achieve fertilization and pregnancy but there is no special 'stem' sperm cell. Noticeably, when mice hematopoietic malignant cells were transplanted in mice recipients, the yields were much higher even tending to one tumor per cell (4,48).

Reviews on melanoma concluded on the existence of melanoma stem cells (49). However, recent work on unselected human melanoma cells shows that by lengthening the observation periods during which the presence of a tumor is investigated and by further depressing the immune response of already immunocompromised mice, the number of cells required to induce a tumor is decreased by five orders of magnitude. In fact under these conditions, more than one out of four melanoma cells induce a tumor (50). Even if we do not take into account these disturbing observations, while xenotransplant experiments suggest that some cells in a tumor have a better ability than others to generate a tumor, nothing proves that this is a qualitative permanent property rather than a transient characteristic of some cells at one moment, i.e. that it defines a qualitatively different population of cells (41).

Although not stated as such, the selectively endowed capacity implies that the CSC, the original cancer cells of a tumor, must *a priori* be endowed with the defining properties, the hallmarks of cancer cells (51), such as self-sufficiency in growth signals, insensitivity to antigrowth signals, resistance to apoptosis, etc, i.e. with asocial and selfish, self-sufficient, competitive properties. Cancer cell lines fulfill those criteria (52–54) and have been proposed as models for CSC (55). Immortality implies telomerase activity at some time, which is indeed expressed at low level in SSC and in cancers in general (56). Adult stem cells have the longest telomeres (57). These properties, to our knowledge, have not been systematically investigated. It should be re-emphasized that these properties are opposite to those of ESC and SSC, which, far from being self-sufficient, require a very specific microenvironment and factors, the 'niche', to survive. However, the default state of the normal stem cell is to limit its life span and to differentiate (58,59), as would be the default state of the CSC.

Second criterion/property: sustaining the hierarchical growth of heterogenous cancer tissue recreating the full repertoire of cancer cells

This is the property directly inspired by the corresponding and established characteristic of the normal embryonic and SSC. Cancer evolution would be a parody of the normal evolution of stem cells

to the sequential hierarchy of pluripotent and terminally differentiated blood or tissue cells (60). An argument in favor of this concept is the fact that undifferentiated, supposedly, CSC containing xenotransplants fully reproduce *in vivo* all the various heterogenous features and cell types of the original tumor. This has recently been reproduced using single cells from cancers with characteristics of CSC (35) but also of common melanoma cells (50). This property implies that there are at least two different populations of cells in a cancer: a minority of originating undifferentiated CSC and a majority of 'derived cells' more and more differentiated and with a limited life span (10). This would be in line with the concept that cancer is essentially a disease of differentiation (61). These properties are always assumed, sometimes clearly stated, and even supported e.g. proposed prostate CSC would not be androgen dependent, whereas derived cells would be (62).

The distinction between a minority of CSC and a majority of derived cells (32) implies that all gross measurements of a tumor property (gene expression, proteome, etc) in fact reflect mainly the majority of derived cells and not the original cancer cells. The remarkable success of gene expression signatures in classifying and predicting the prognosis and response to therapy of many tumors (63) would bear against such a conclusion unless the signatures of derived cells merely reflect those of the CSC.

The mechanisms and processes involved in the generation of the derived population are rarely discussed. They are often implicitly assumed to be similar to those of the SSC. In the latter case, this entails an asymmetric division generating, by a qualitative deterministic process, one daughter stem cell and one more differentiated derived cell. In SSC, this process is physiologically irreversible. Let us examine how these stem cell properties apply to CSC (Table I).

There is no evidence of asymmetric divisions of cancer cells and in fact the possibility that such a remarkable evolutionary process could be co-opted in CSC looks improbable. In the SSC, the differentiation of the derived cell is deterministic and qualitative; there are no quantitatively modified intermediates. In the case of CSC, this has not been proved (64). The physiologically irreversible character of the derived population also remains to be proved. There are arguments for both: aggressive CD133+ ovarian cancer cells generate less aggressive CD133− cells and not conversely unless with a treatment with epigenetic inhibitors (of DNA methylase and histone deacetylation). However, the fact that supposed markers of CSC are in fact properties modulated by physiological or pharmacological agents bears against this concept. The number of spheres generated from a set of cancer cells increases in response to epidermal growth factor (53); the expression of the CD44 biomarker is highly modulated by several signal transduction pathways (65,66); properties of CSC are acquired as a consequence of induced epithelial mesenchymal transition in cancer cells (67). Moreover, some reversibility has also been demonstrated in SSC (supplementary Information 3 is available at *Carcinogenesis* Online).

The constancy of the CSC fraction (in fact ABCB5 positive cells) from the parent tumor to the serially passaged xenotransplants, e.g. in melanoma (29), would be difficult to explain without a sophisticated homeostatic control such as those operating in SSC. The various rates of proliferation and differentiation would have to be neatly adjusted to avoid the dilution of CSC or conversely their overtaking of the tumor. This is explained in the case of SSC by a tight control of the microenvironment, the niche, which maintains the SSC population, while displacement outside causes terminal differentiation, and later the loss by shedding of the differentiated derived cells. Modeling could help to understand and define the constrains that would have to be met to sustain an equilibrium between the two sets of cancer cells. Such an equilibrium would be even more difficult to achieve in a cancer cell line propagated serially.

Third criterion/property: expression of a distinctive repertoire of surface markers and other characteristics

The ultimate proof of the existence of a distinct subpopulation in a cell population is to isolate and characterize it. For this, repertories of biomarkers and other specific characteristics have been proposed for CSCs and demonstrated for SSC. The most convenient biomarkers are those that allow antibody-directed cell sorting i.e. membrane biomarkers. In breast cancer cells, active aldehyde dehydrogenase 1 also allows cell sorting thanks to the use of a fluorometric product of the enzymatic reaction (the Aldefluor test) (68). Several biomarkers have been proposed; some common to different cancers, but no universal marker for CSC has yet been identified (42,69) (see supplementary Information 4 is available at *Carcinogenesis* Online). If the relative specificity of these expressions is accepted, their demonstration in whole tumors is hardly compatible with the hypothesis that they would characterize only a minor population of CSCs.

Cells resistant to chemotherapeutic drugs are endowed with ATP-binding cassettes ABCB (e.g. ABCB5, ABCB1, ABCC1 and ABCG2) ATP-dependent macromolecules transporters that expel xenobiotics and drugs from the cell. These cells, contrary to other cells, do not accumulate Hoechst 33342 dye, as they expel it, which allows these cells to be separated by cell sorting. This pumping out is inhibited by verapamil. If it is assumed that these cells are the postulated CSCs, it is a convenient way to separate them. A category of ovarian cancer cells defined by this criterion is highly proliferative, poorly apoptotic and responds remarkably to an eradicating interferon α treatment (27). However, the dye is toxic and dye exclusion allows to avoid this toxicity and thus, may confer to these cells an acquired advantage in later growth (22).

A proposed functional characteristic of CSC is their ability to grow in tumorospheres, analog to neurospheres and to mouse embryonic fibroblasts in suspension. In some cases, these spheres incubated in the absence of added serum present a massive apoptosis of some cells (derived?), whereas the others (CSC?) proliferate. Thus, sphere culture is one of the methods used to enrich cancer cell populations in CSCs. However, sphere cells are heterogenous and only a fraction of them have the properties of CSCs. Disturbingly, these fractions vary with passages (42). This could explain the wide variability of quantitative data in the literature. Of course, the CSCs, as the SSC, would be undifferentiated and therefore devoid of differentiation markers, but their derived cell population would probably reacquire some of these properties.

In fact, most authors define CSC as the cells they isolate using their own favorite biomarkers. If these biomarkers characterize one population of cells, one would like to see that each subpopulation of cells isolated using one criterion would also present the other properties (40) and sequential application of the various criteria should not decrease markedly the estimated CSC fraction of a population. This is rarely done and when it is, the overlap is only partial (10,28,43,70). Colorectal cancer cells that display both CD44 and CD133 markers are clearly distinct (71), but only CD44 expression seems essential for sphere and xenograft development. There are other examples (72) (supplementary Information 5 is available at *Carcinogenesis* Online). The fact that CSC markers are also expressed in other cells, as well as the fact that these markers are induced and repressed in various conditions, does not fit well with the concept of a fixed qualitatively well-defined cell population (see supplementary Information 6 is available at *Carcinogenesis* Online).

An implicit assumption of the biomarker concept is that, in a given cancer, these biomarkers are well defined, qualitative and permanent which suggests that the quantitative distribution of biomarkers in the population would be all or none and not continuous. Most of the data seen on cell sorting do not show this.

It would of course be satisfying if a role of the biomarkers in the biological characteristics of the CSC could be demonstrated. However, apart from the dye exclusion property, which would explain the chemoresistance of CSCs, their persistence and resurgence after treatment, the role of the biomarkers in the properties of CSCs has been little studied (supplementary Information 7 is available at *Carcinogenesis* Online).

In conclusion, biomarkers, including membrane biomarkers, but also functional biomarkers such as growth in spheres or chemoresistance, allow to enrich cancer cell population in more aggressive,

reproducing, enduring and tumor-generating cells, the CSC. However, most of these biomarkers seem to be much more prevalent in the tumors than specific expression in a restricted cell population would allow. Congruence of these biomarkers and their permanence in specific cells remains to be demonstrated. The biological role of these markers represents an interesting question. Independently of their proposed CSC specificity, biomarkers expression may constitute useful clinical prognosis indexes (73).

Other properties ascribed to CSC

An important property of CSC, not included in the definition but related to their selective capacity, is their resistance to chemotherapy and to radiation. Such a property might result from several causes: an ABC transporters overexpression, a high capacity for DNA repair, a low proliferation rate, a resistance to apoptosis, a reduced immunogenicity, etc (42). It opens the possibility of devising treatments specific to such cells and thus to prevent relapses and cure cancers (10,36,43,74–76). However, again there is little argument as yet that these properties are qualitative rather than quantitative. Noticeably, this property may be shared by SSC as suggested for bone marrow, gut mucosa (32) and for hair recovery after a loss induced by X rays or cancer chemotherapy.

The resistance to radiation of CSCs is controversial (74,77). Such a property and the resistance to chemotherapy might be explained, at least in hematopoietic stem cells, by the induction of a p21 cellular response which leads to reversible cell cycle arrest and DNA repair (78). Also these cells may have a higher resistance to reactive oxygen species because of an increased expression of scavenging systems (79). Conversely, ovarian CSCs would be more sensitive to interferon γ (27).

Some characteristics of SSC obviously do not apply to the CSC. The homeostatic control which maintains the size and composition of intestinal acini, hair follicles and their niches (58), etc, has obviously no correspondence in cancer, although a symbiotic relation between CSCs and endothelial cells has been proposed (80).

Cell multiplication in CSC is controversial. Various types of cell multiplication models exist (Figure 1). The division rate of stem cells varies, depending on the type of stem cells: low in most SSC and high in ESC (26). For CSC, some authors postulate or find a low division rate as in SSC, others a rapid rate (12,22,27,28). Some others postulate the existence of dormant and cycling CSC (81). A low division rate would explain the high dilution of CSC in the cancer cell population and, in part, a greater resistance of CSC than derived cells to chemotherapy and radiation (9,78). However, in a rapidly growing population, such cells could then be outcompeted. The mathematics of the evolution of the proportion of CSC versus derived cells is far from evident (9,82) but they suggest that if CSC exhibit some stem cell characteristics, they would be those of ESC, with their rapid multiplication rate, rather than those of SSC that divide infrequently.

CSC and cancer cell lines

The concept of the existence of a minor subpopulation of CSC in cancer cell lines (9,30,75) and the alternative of whole cancer cell lines as a model for CSC (55,83) have been proposed (supplementary Information 8).

Conclusion

The analysis of proposed properties and implicit assumptions about the CSC concept shows that, whereas the heterogeneity of tumors with cancer-propagating cells (the CSC?) and derived more or less handicapped cells (derived cells) seems well established, the existence of a single, unique and homogenous CSC population of these cells is highly improbable (40,84,85) and the qualitative, deterministic and irreversible characters of the transformation of the first population are questionable or at least remain to be proved. In any case, the cancer-propagating cells are very different from normal stem cells, which make the CSC terminology inadequate and misleading (see Appendix for terminology). Of course, these tumor cell types can

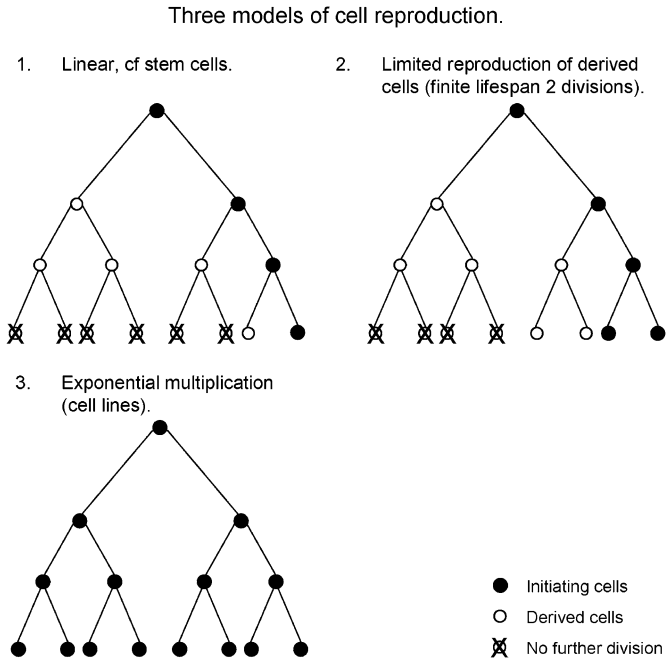

Fig. 1. Models of cell reproduction. Linear: fixed population of stem cells generating derived cells with limited life and life span: constant population (Example: SSC and derived cells). Limited reproduction of derived cells, expanding reproduction of stem cells and generation of derived cells with limited life and life span: growth. The CSC concept. Exponential: homogenous population of reproducing cells with extended life span (infinite?): exponential growth (Example: cancer cell line in culture).

be as or more diverse as SSC types. In this case, each one will have to be characterized and the present definition should be considered as the common hat covering diverse situations. The number of published articles on the subject reflects its ambiguities.

Alternative to the standard CSC model

There is no doubt that cancers are heterogenous and contain more and less competitive cells. This is illustrated by the importance of cell waste in human solid tumors and the diverse effects of mutations.

First, the importance of cell waste in human solid tumors. By investigating cell kinetics in fragments of mammary cancers, Tubiana could evaluate the doubling time and thus the proliferation rate of mammary cancer cells. When compared with the real measured volumetric development of the tumors, a gap of up to 90% was observed. Obviously, the rate of wasting is very high. Thus, tumors lose a lot of cells (86). They can also lose reproductive cells by differentiation and senescence (87). Indeed, oncogene-induced senescence must constantly be suppressed in cancer cells (87,88).

Second, the diverse effects of mutations. Tumor cells are constantly and heavily mutagenized (89). The total number of mutations in any cell is therefore probably much larger than the common set found in bulk cancer DNA (90). In cell populations exposed to mutagens, the majority of the mutations are neutral or silent (90%), a sizable proportion (9%) is deleterious and only a minor fraction (1%) confers a competitive advantage (91,92). Mutations conferring an advantage would be those stimulating self-renewal, i.e. proliferation, inhibiting differentiation or suppressing apoptosis, negative feedbacks and external controls, i.e. mutations conferring to the cell the hallmarks of cancer cells. Aneuploidy has similar consequences (93). In the competitive environment of a tumor, all impaired cells, e.g. by a loss of telomerase activity, will disappear at some time, whereas the few more aggressive cells will thrive (11). The same reasoning can be made for epigenetic effects. Tumors escape the effect of

ErBB2-targeted drugs by similar stochastic mechanisms (94). Thus, in a tumor cell population, one could expect a large continuous spectrum of tumor cells from the most competitive to the disabled or doomed cells. Such heterogeneity would promote tumorigenicity (95). As shown by the finding of common mutations in bulk cancer DNA, this will be limited: random genetic or epigenetic alterations will lead to increased variance but selection will decrease it (Figure 2).

The question then is what are the competitive cells and which is their evolution. We and others (7) have ruled out the possibility of a single unique homogenous population of irrevocably determined cells, the CSCs.

In biology in general, and in cancer in particular, nothing makes sense without Darwinian evolution (96). One cancer has even evolved to transmissibility between dogs, i.e. to immortality (97). The analogy with humans who all bear the same mitochondrial DNA ('Eve') is striking. In humans, a similar congruence testifies to one or several bottlenecks in evolution and fierce elimination of the other genotypes. This is akin to antibiotic resistant bacteria that also outcompete all others in epidemiology. A similar evolution can be postulated for the cancer cells. At each stage in the mutation sequence, the cell that has acquired a new advantage supersedes the others. Intermediates with less mutations must also have had a competitive, albeit a weaker, advantage to be available for further mutation. The fact that intermediates have disappeared may in part reflect the effect of deleterious genetic or epigenetic events but mostly it suggests elimination by competition (98–100), which is a very general cell process (101). The less competitive cells originating from the present 'driving mutations cells' (TPC) could represent the derived cells. This implies that, contrary to the SSC, the TPC must have outcompeted all others while still generating derived cells, i.e. that they have a much higher reproduction rate. In this scheme, very competitive clones might and probably have evolved separately, certainly in distinct parts of a tumor. In the absence of such clones, the cancer may often not evolve: most cancers do not achieve an aggressive form (102).

The sequential model of progressively evolving populations of more and more aggressive descendents of initial tumor cells is supported by the genetic analysis of cell lineages of a mouse tumor (103) and by the clonal progression through sequential acquisitions of genetic alterations in cell lines (76) and in human thyroid papillary carcinomas (104). It may also result from epigenetic (105) and even non-genetic 'lysogenic-like' (106,107) mechanisms (see Appendix for terminology).

Around 80 mutations are demonstrated in individual cancers (108,109). Some may be driver mutations, others passenger mutations (110,111). The mere fact that such mutations can be found by global sequencing of DNA from a piece of cancer tissue, comprised of millions of cells, implies that at least a large minority of these cells bear these mutations i.e. that they originate at some time from one selected cell that has acquired them over many generations which later have evolved and outcompeted other cells. It illustrates the strong convergence resulting from a Darwinian evolution (Figure 2).

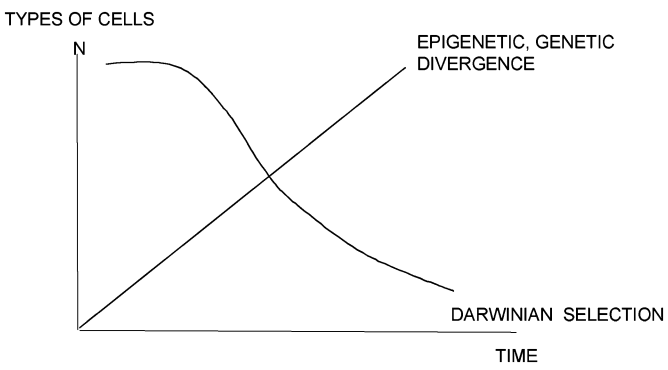

Fig. 2. Opposite effects of divergence and selection on the diversity of cell population in a cancer.

Tumors are therefore probably constituted by a majority of less competitive, severely handicapped or doomed cells and a minority of more competitive cells. The latter ones will to some extent behave as expected for CSCs. However, such cells would not constitute a unique homogenous but a heterogenous population. They would be constituted of cells of the same origin having diverged and multiplied at different times in the evolution of the tumors and having survived a fierce competition. They would be stochastically and not deterministically selected (89) i.e. by a Darwinian selection (96). They could acquire some stem cell properties (112). They would be quantitatively rather than qualitatively different from the derived populations (113). There would be no fixed irreversible barrier between the two populations but a difficult but not impossible reversion to a more competitive state (42). The state of these cells needs not to be permanent; cells could be more competitive in one localization or at one time. Slow non-synchronized fluctuations that underlie the heterogeneity of even clonal populations (106,114) could partly account for the diversity. The mitogenic response of dog thyroid cells to stimuli *in vitro* is very heterogenous in time, which shows that the individual cell proliferation status fluctuates with time (115). Even in classical stem cells, the loss of 'stemness' is not necessarily irreversible (vide supra).

There are many arguments in favor of the concept of a dynamic equilibrium with some reversibility in transitions between cancer cell populations (116) (Figure 3) (supplementary information 9 is available at *Carcinogenesis* Online).

There could be different sets of competitive cell types with overlapping properties (98,117). For instance, in a colon cancer cell line, CD133 and aldehyde dehydrogenase 1 expressions have a synergistic effect on *in vivo* tumor potential (118). In fact, the existence of additive or multiplying tumorigenic potencies in cells expressing one or the two markers CD133 or aldehyde dehydrogenase 1 favors a model of continuous rather than all or none tumorigenicity (Figure 4). Any of these cells could reinitiate a full tumor (35). This is compatible with a model of random genetic, epigenetic and functional lysogenic like, oncogenic events striking a population, the addition of which could confer phenotypes with great competitive advantage, general or local, to some cells, which would become the driving elements in the dynamic evolution of cancer and would outcompete the others (119). Different genetic or epigenetic events affecting different competitive cells at any stage could lead to new clones. While genetic events would only be reverted by other antagonistic events (e.g. an inactivation downstream from a constitutive activation), epigenetic and lysogenetic events are reversible. The marked heterogeneity of genetic events in different regions of some thyroid papillary carcinomas and in human glioblastoma (120,121) and between original tumors and some metastases (122) is compatible with this. For other cancers, this is discussed at length in a recent review (123). Although reasoning in the framework of the CSC concept, Siegmund *et al* concluded from a study of DNA methylation patterns in colorectal cancers to the existence of 4–1000 CSC lineages per cancer (124). A stochastic dynamic model within the colon cancer CSC populations has been suggested on the basis of the existence of different consistent and unique chromosomal aberrations in these populations (125). The dynamic model is compatible with the clonal spatial diversity of tumors (126) and with the coexistence of different lineages in relapsed acute lymphoblastic leukemia (127). It better fits the findings that >25% of melanoma cells are able to generate melanomas in severely immunocompromised mice and that this property has no relation to the expression of biomarkers (50). It also fits with the fact that most cancers do not even achieve an aggressive form (102) for lack of the necessary ultimate transforming events.

Such a concept would explain the only partial overlap of CSC different properties. The fact that markers are only expressed together in the so-called CSCs but that other cells share differently some of these markers, as well as the easy induction and repression of these markers would also support the concept of a dynamic, quantitatively

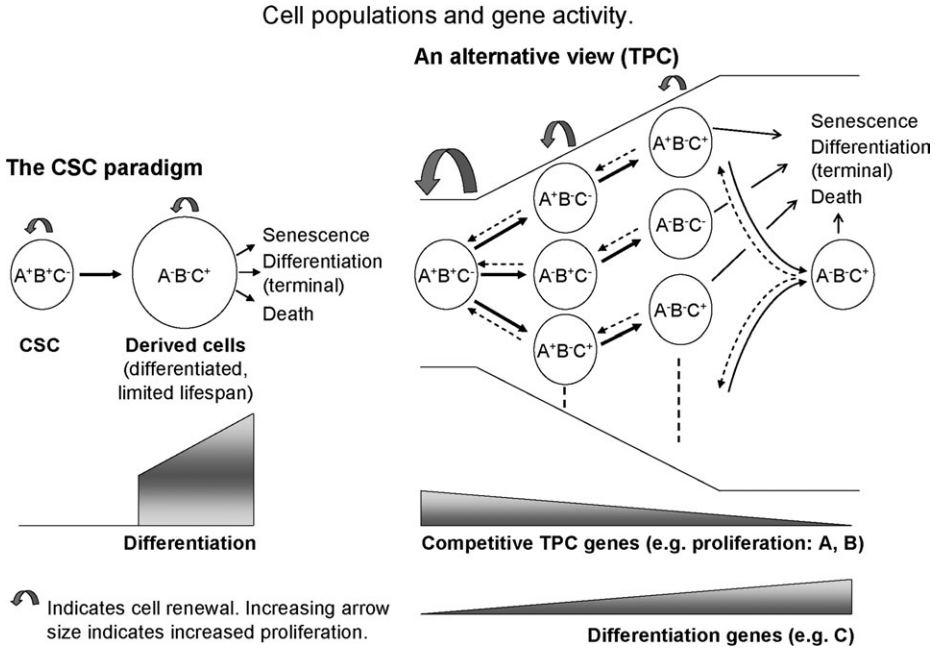

Fig. 3. Concept of the CSC and an alternative view (TPC). $A^+B^+C^-$: necessary properties of the CSC^- TPC lost in derived cells. C^+ property acquired in derived cells. CSC one homogenous population, self-reproducing, transformed irreversibly in a derived population with various fates. In the alternative model, independent, partially reversible properties conferring a competitive advantage. In this model, cells having lost these properties are outcompeted and end up in terminal differentiation, senescence or death.

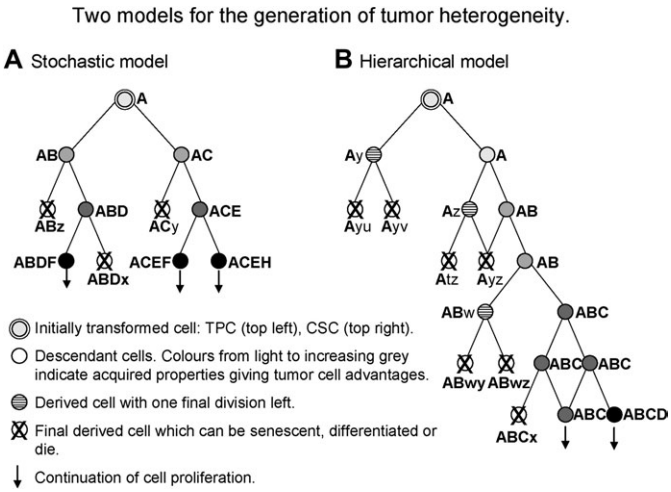

Fig. 4. The two extreme concepts of cancer evolution. (A) The stochastic model in which successive functional, genetic or epigenetic events affecting the population of cells will lead to more and more diversified, more or less competitive cells. The scheme does not include the Darwinian selection that will restrict drastically population diversity. (B) The hierarchical model as generally presented, with an evolving CSC population with some symmetrical divisions and many irreversible asymmetric divisions generating derived cells with limited life spans. This scheme is derived from the only such representation that we found (44) and which is just reproduced in later reviews. ABCDEF … represent the progressive addition of heritable changes increasing competitivity; wxyz are deleterious genetic or epigenetic events. Whatever the model retained, it should be emphasized that the number of events involved to achieve the fully transformed cell needs not be very high considering that only the acquisition of a limited number of properties is necessary and that any event may involve the taking over of a whole program enhancing the chances of propagation (e.g. cell proliferation ESC program, epithelial mesenchymal transition, etc). The expression of only four transcription factors is sufficient to recover an ESC from a differentiated skin cell!! To simplify the figure, derived cells show no further division (left) or one further division (right) that is arbitrary.

different TPC population (Figure 4). Such a model would also explain how one can obtain supposed CSCs from homogenous cancer cell lines. One consequence of the model is that it makes sense to investigate biomarkers not only as such but also as characters that confer a competitive advantage, the combination of which makes the most dangerous cancer cells.

The CSC and the stochastic quantitative partially reversible models are not totally exclusive of each other (35) (Figure 4). In fact, the CSC model is just an extreme case of the stochastic model. The main questions to be answered concern the heterogeneity of the propagating cells, the properties of stochasticity versus determined program, reversibility, genetic versus epigenetic or functional and qualitative versus quantitative nature of CSC characters. Further investigation is necessary to establish to what extent a model will account for all the facts and to what extent the same paradigm will apply to different cancer types. Variants of both models could exist in different cancers or even coexist in the same cancers (35). The two models are compatible with a clonal, i.e. genetic evolution of tumors that would involve at one extreme the CSCs or at the other all the competitive tumor cells (43,128). These models do not exclude the possibility that, like in bacterial populations (129), there could also exist fully developed but transiently dormant driving cancer cells, poorly sensitive to chemo or X ray therapy, that could revive, randomly or in response to important cell loss, and cause cancer relapses.

Whatever the definition that will emerge from further studies, many questions remain (supplementary Information 10 is available at *Carcinogenesis* Online).

Whatever the future definition of CSC and TPC, the basic idea of using various relatively specific properties of the more competitive cells, such as their high or low proliferation rate (47), their resistance or sensitivity to chemotherapy or radiation to prevent or cure tumor relapses should be retained even if the dogma of CSCs is modulated (43). However, the concept that we shall retain of the CSC–TPC has clinical consequences. If a tumor develops from a homogenous but scarce population of CSC it makes sense to search for the Achille's heel of such a population for treatment. On the other hand, if the CSC

are heterogenous and evolving as we propose, it would make more sense to attack simultaneously several different populations and targets at the same time i.e. a polytherapy analogous to the now standard method of treating HIV.

Supplementary material

Supplementary Informations 1–10 can be found at http://carcin.oxfordjournals.org/

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Appendix

Terminology

CSC or TPC.

Individual cancers, although they bear in the majority of their cell constituents a common large set of mutations, are constituted of cells with different abilities to generate tumors in xenotransplanted animals. The best tumor-generating cells are commonly called CSC, the others the derived cells. This term suggests that the cancer cells originate from SSC, that they share properties with them, and that they constitute a distinct population of cells qualitatively and irreversibly different from other derived cells. As such assumptions are debatable and often only partially true, the term is both inappropriate and misleading. Other more suitable terms have been proposed: TIC and stemloids (83). The term stemloid acknowledges some similarities with real stem cells but implies that they are in fact different. TIC is an operational term designating in the population of cancer cells those that are able to generate a transplant in an immunocompromised animal. The TIC terminology introduces a confusion suggesting that these are the cells that have initiated the tumor *in vivo*. As in fact the cells we consider are defined by their presence in existing cancers and their ability to propagate *in vitro* and *in vivo*, we retain the strictly operational term: TPC proposed by Kelly *et al.* (4) and Hong *et al.* (5).

Cell hereditary mechanisms.

(i) We call genetic, the cell hereditary features resulting from mutations, i.e. modifications of the coding sequence of DNA (e.g. point mutations, insertions, deletions, recombinations, etc).
(ii) We call epigenetic, the cell hereditary features resulting from covalent modification in chromatin structure (DNA methylation, histone methylation, acetylation, phosphorylations, etc).
(iii) We call functional ‘lysogenic like’, the transmissible states resulting from signaling systems exhibiting only two exclusive states such as resulting from regulation involving positive feedbacks in gene transcription especially coupled to positive cooperativity (e.g. for bacteriophage, colonization of bacteria with either lysogeny or lysis). Such mechanisms could play a role in Darwinian evolution provided that the non-genetic variant cell maintains its distinct status for a sufficiently long period of time and transmits it to daughter cells, as in lysogenic bacteria (106,130).