

Stem Cells and Cancer

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Introduction

The cancer stem cell represents a useful model – increasingly supported by experimental findings – to explain and investigate cancer. In general, cancer is thought to arise from normal tissues along a multi-step progression from precursor lesions to increasingly more invasive (malignant) stages. Local and distant metastases arise from these primary malignant lesions and still represent one of the major causes of morbidity and mortality among cancer patients throughout the industrialized world. Along this sequence of events, a stepwise accumulation of genetic alterations in specific cancer genes is considered the driving force in tumor initiation, progression and metastasis.

A well-defined number of cellular changes, such as self-sufficiency in growth signals, resistance to programmed cell death (apoptosis), insensitivity to growth-inhibitory signals, limitless replicative potential, and the capacity for inducing growth of new blood vessels (angiogenesis), are thought to represent essential requirements for the cancer cell to grow and invade distant sites.⁽¹⁾ However, although formally correct, this model takes little account of other essential characteristics of human cancers, namely their pronounced cellular heterogeneity (many different cell types are often present within the tumour mass) and the putative role played by a subpopulation of cells, the cancer stem cells (CSCs), in driving tumour growth and determining local invasion into surrounding tissues and distant metastasis.^(2,3)

Tumours are not autonomously-acting proliferation machines, but are very heterogeneous, both in their morphological and functional aspects. In fact, an individual tumour may show distinct areas of proliferation, cell cycle arrest, epithelial differentiation, cell adhesion and dissemination. According to this more dynamic CSC model (**figure 1**), the majority of tumour types arise from within stem cell niches characterised by a tightly co-ordinated balance between self-renewal, migration, proliferation, differentiation and apoptosis. Mutations in genes known to be responsible for this balance in normal tissues result in the formation of a partially differentiated and heterogeneous tumour mass that, upon additional mutations and under the positive influence of micro-environmental factors, progresses towards malignancy.

Tumour cells are shed from this heterogeneous mass into the micro-environment. However, they will reflect the heterogeneity of the primary tumour and only few, the *migrating* cancer stem cells, will retain the necessary plasticity to undergo trans-differentiation and enable their migration and homing in distal organs.⁽³⁾ Accordingly, aggressive cancer progression has been correlated with the loss of epithelial identity and the acquisition of a migratory phenotype. This phenomenon, referred to as *epithelial to mesenchymal transition* (EMT), is considered a crucial event in malignancy. Additional steps enabling dissemination and metastasis may be reversible (mesenchymal to epithelial transition, MET), and thus cannot solely be explained by irreversible genetic alterations, indicating the existence of a dynamic component to human tumour progression and of a regulatory role for the tumour environment.

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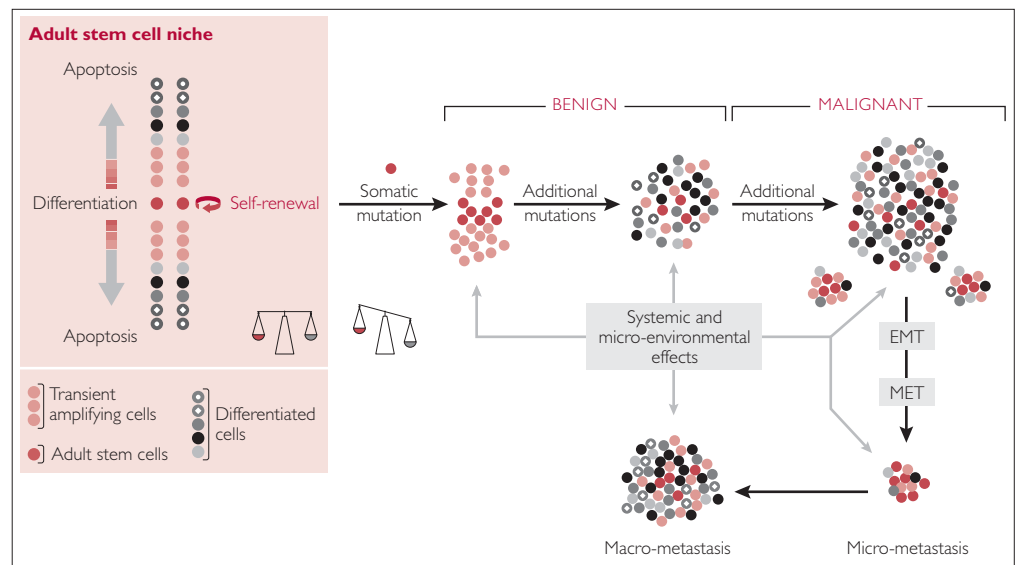


Fig.1 Schematic representations of the cancer stem cell hypothesis in tumor formation and progression to malignancy. In the adult stem cell niche, pluripotent stem cells together with more committed progenitors and fully differentiated (specialized) cells are kept in equilibrium. Gene mutations leading to cancer alter this equilibrium between stem cells and more specialized ones. This disequilibrium results in excessive cellular proliferation and/or to lack of programmed cell death (apoptosis) thus leading to the formation of a heterogeneous cellular mass (benign tumor). Additional gene mutations underlie progression to more malignant tumor stages. Different cellular types will detach from the primary tumor, reflecting its heterogeneous cellular composition. Cancer stem cells, because of their plasticity and capacity to trans-differentiate, will successfully move across the body and invade distant organs by forming micro-metastases (enriched in cancer stem cells) and eventually macro-metastases which recapitulate the primary tumor in terms of cellular composition and heterogeneity.

To summarize:

- Tumour heterogeneity is not explained by current genetic models for tumour initiation and progression to malignancy and metastasis.
- CSCs arise from their normal counterparts within adult stem cell niches.
- CSCs represent a minor, though highly relevant, sub-population within the tumour mass.

Current status

An important area of research is tumour-specific CSC surface markers underlying molecular and cellular defects. The concept of cancer stem cells was first proposed more than a century ago but only recently has it gained new momentum with the latest advances in stem cell biology. Normal stem cells are commonly defined by two main intrinsic features: the capacity of perpetuating themselves (through self-renewal), and that of acquiring and exerting specialized

functions (through differentiation). Self-renewal and differentiation are inherent to the stem cell's ability to divide 'asymmetrically', meaning that each cellular division can generate both pluripotent (stem-like) and committed (differentiated) daughter cells in a strictly regulated fashion.

Apart from their embryonic counterpart, 'adult' stem cells exist, albeit at low level, in virtually all organs of our body and play essential roles in preserving and replenishing tissues in our adult body. Moreover, specific stem cell niches are expanded upon hormonal stimuli (e.g. the mammary gland during pregnancy) or recruited to repair tissue damage (e.g. during wound healing). Within these adult stem cell niches, it is of utmost importance that self-renewal and differentiation, but also cell migration and programmed cell death, are strictly kept in equilibrium so as to avoid either cellular loss (tissue waste due to more differentiation than self-renewal) or gain (tissue growth or neoplasia, due to more self-renewal than differentiation).

Cancer stem cells are likely to arise from their long-lived normal counterparts through muta-

Cancer Type	CSC-specific markers	Reference
Leukemia	CD34 ⁺ /CD38 ⁻	<i>Nat Med</i> 3 : 730 (1997)
Breast	CD44 ⁺ /CD24 ^{low lin-}	<i>PNAS</i> 100 : 3983 (2003)
Brain	CD133 ⁺	<i>Nature</i> 432 : 396 (2004)
Myeloma	CD138 ⁻	<i>Blood</i> 103 : 2332 (2004)
Prostate	CD44 ⁺ /α ₂ β ₁ ^{hi} /CD133 ⁺	<i>Cancer Res</i> 65 : 10946 (2005)
Lung	Sca-1 ⁺ /CD45 ⁻ /Pecam ⁻	<i>Cell</i> 121 : 823 (2005)

Tab. 1 Prospectively identified cancer stem cells and the corresponding markers

tion events affecting signal transduction pathways (e.g. Wnt, Hedgehog, and Notch) known to regulate this finely regulated balance. The latter can be achieved, for example, by simply favouring symmetric vs. asymmetric cell division or by insensitivity to growth-inhibitory signals from the surrounding stem cell niche.

Hence, according to the CSC model, a small sub-population of cells retains stem-like properties and is responsible for tumour growth (by self-renewal) and heterogeneity (by differentiation). Experimental evidence for the existence of cancer stem cells has been delivered for several tumour types including leukaemias, multiple myeloma, breast, brain, prostate and lung cancer (table 1). In this approach, human tumours are dissociated to single-cell suspensions, sorted by different cell surface markers, and transplanted into immunodeficient (NOD/SCID) recipient animals.

Cancer stem cells are hereby defined by their capacity to recapitulate tumorigenesis even when transplanted at low multiplicity in an experimental model. For example, 1 in 10⁵ acute myeloid leukaemia (AML) cells express the cell surface markers CD34⁺CD38⁺ and can recapitulate the histological heterogeneity of the disease when transplanted in SCID animals, whereas CD34⁺CD38⁺ leukaemic cells cannot, even at higher multiplicities. Likewise, human breast cancers encompass a subpopulation (1-10 per cent) of CD44⁺CD24^{low lin-} cells. These putative breast CSCs can form tumours in NOD/SCID mice when as few as 200 cells are transplanted. Notably, many cell surface markers are

shared by CSCs isolated from different cancer types, possibly indicating activation of common signal transduction pathways. Deregulation of the Wnt signal transduction pathway for example, has been shown to be a very early event in colon, breast, skin and haematopoietic malignancies and is likely to activate self-renewal and ‘stemness’ in the corresponding tissue-specific niches^(4, 5)

To summarize:

- Self-renewal and differentiation represent the main CSCs’ features: the former drives tumour formation and growth whereas the latter underlies tumour heterogeneity.
- Deregulation of signalling pathways known to modulate self-renewal and differentiation during development and in adult stem cell niches underlies CSC onset and invasive behaviour.
- Cell-sorting by specific combination of cell surface markers and transplantation in immune-deficient mice has allowed the prospective identification and isolation of CSCs from different human cancer types.

Prospects

Research on cancer stem cells offers prospects for improvements in cancer prognosis and treatment. The demonstration of the existence in several human malignancies of a minority of cancer stem cells capable of reproducing the heteroge-

neity and malignancy of the human disease in experimental animals has several implications for cancer prognosis and treatment. Expression profiling is currently regarded as the most promising tool for cancer prognosis and response to treatment. However, if tumour onset and invasive behaviour are driven by a minority of CSCs, their relative number within the tumour mass may correlate with the patient's risk of developing local and distal metastases. Hence, expression profiling of total tumours by microarrays encompassing the complete set of human genes is unlikely to detect CSC-specific expression signatures as they are diluted within a majority of heterogeneous cellular types. Likewise, whole tumour expression profiles are likely to be of less value to design tailor-made therapeutic approaches.

The identification of CSC-specific signatures by expression profiling of purified CSCs will allow their supervised bioinformatic analysis from whole tumour profiles and the refinement of clinical prediction of metastatic behaviour and response to treatment. Also, it will facilitate the identification of novel CSC-based therapeutic targets. Upon conventional chemotherapy or radiation therapy, tumour shrinkage is likely to result from death of differentiated tumour cells. However, if CSCs represent a minority of cancer cells and if, as reported in the literature, they are characterised by an intrinsic resistance to radiation and chemical agents, they are likely to escape adjuvant therapy and underlie cancer relapse. Therefore, the development of therapies specifically targeted against CSCs is likely to result in considerable improvement of the cancer patient's long-term survival.

The cancer stem cell hypothesis also suggests novel prospects for the detection of circulating cancer cells after surgical removal of the primary malignancy. In general, circulating cancer cells in blood and bone-marrow are known to be present at various multiplicities in cancer patients. Circulating cancer cells however, will reflect the heterogeneity of the primary tumour mass and only a minority will succeed in the metastasis of a distal organ. CSCs, because of their intrinsic plasticity and capacity of transdifferentiate upon stimuli from their direct environment, represent a clinically highly relevant sub-population of migrating cancer cells capable of reproducing

the primary lesion at distal locations. The identification of CSC surface markers for specific cancer types (**table 1**) will allow the detection and quantification of migrating CSCs in body fluids, blood and bone marrow, and guide post-surgical clinical management and surveillance of the cancer patient.

To summarize:

- Whole tumour expression profiles are likely to encompass heterogeneous cellular types thus possibly masking the clinically relevant CSC sub-population.
- Definition of CSC-specific gene signatures will improve our capacity to predict cancer prognosis and response to treatment based on expression profiles of whole tumours.
- The same CSC expression profiles will facilitate the identification of therapeutic targets for tailor-made intervention.

Conclusions

The cancer stem cell hypothesis represents a truly innovative concept in cancer biology with profound fundamental and clinical implications. Research on cancer stem cells will run in parallel with the research on normal embryonic and adult stem cells. Central to both lines of investigation is the identification of cell surface markers for their prospective identification from healthy and diseased tissues. The subsequent molecular characterisation of the purified stem cells by genomic and proteomic analysis will lay the foundation for the elucidation of the molecular and cellular mechanisms that regulate self-renewal and differentiation in homeostasis and cancer. These advances in our understanding of cancer stem cell biology will open new avenues for the improvement of cancer risk assessment, prognosis, surveillance, prevention and targeted therapy. To this end, both fundamental and applied research should be encouraged, combining genetic, cellular and molecular analysis of *in vitro* and *in vivo* CSC experimental models with the prospective detection, purification and analysis of CSCs from surgical specimens and body fluid from cancer patients.