Controversies regarding cancer stem cells

JLM Ruiz1, D Levy1, SP Bydlowski1*

Abstract

Introduction
The process of oncogenesis involves an accumulation of rare mutational events, or hits, that lead to altered control of cell growth, death, and differentiation. In the last few years, the theory that cancer may originate from mutations in stem cells has gained a lot of followers. Some researchers have isolated and characterized cells from tumours that were considered cancer stem cells. The discovery and understanding of these cells has the potential to improve treatment outcomes. Whether these cancer stem cells, which are capable of initiating and sustaining tumour growth in animal models, really exist in all cancers is yet to be determined. Here, we discuss the current knowledge regarding cancer origin and cancer stem cells.

Conclusion
The cancer stem cells remain a theory that can help to explain some features of cancer but not all. Still is necessary research to well characterized and compared the cells isolated of different tumours.

Introduction
With the increased human lifespan in the last few decades, cancer has become a more serious issue in medicine. For years the clonal evolution of cancer was the predominant explanation of its origin, and currently the idea that cancer progresses through multiple stages in a unique cell prevails among oncologists. One reason for this view is that, in experimental animal studies, cancer arises after sequential application of different chemical carcinogens. Another reason is the observed age-onset patterns of cancer, in which the incidence often accelerates with age in a manner that suggests progression in multiple stages. These multiple hits can occur in any somatic cell exposed to carcinogenic agents, but it is more probable that they occur in a long lived undifferentiated cell that is able to support and survive these mutational events, such as stem cells (SCs). The clonal growth of the mutant cell is substituted or complemented by the capacity of the SC to differentiate into several cell types (Figure 1). Therefore, the longevity of SCs makes them susceptible to the accumulation of genetic and epigenetic damage in such a way to make them good candidates for the emergence of the neoplastic transformation.

On the other hand, a bulk of evidence supports the existence of a functional hierarchy among tumour cells, and that the existence of treatment-resistant tumour cells can explain the kinetics of disease relapse in mice and humans following chemotherapy. Only after the use of molecular biology techniques could it be established that the immature malignant cells can differentiate in vivo to form more mature-appearing progeny, some of which expressed genes of drug resistance. Another observation is the heterogeneity of tumours (different morphology and gene expression), even among tumours cells from the same tumour or the same cancer type in different patients.

The characteristics of tumour cells described above correlate with SC properties. Stem cells are undifferentiated cells that can self-renew, proliferate, and differentiate to constitute new tissue or fix some tissues. Several types of SCs are known, including embryonic SCs, which exhibit the capacity to differentiate into any cell and form all tissues in the organism, and progenitor SCs, which can form only some specialized cells, such as blood cells. In the last decade, SCs were found and isolated from the embryo and adult tissues, including bone marrow, amniotic fluid, Warthon’s jelly, and adipose tissue, among others. These findings support the idea that each tissue has its own SC reservoir. SCs may divide into two daughters cells with the same phenotype or one cell that retains the same undifferentiated state and another cell that differentiates into a more mature cell, losing the stemness.

Understanding the biology of SCs was fundamental to the advancements that support the idea that some tumour cells originate in malfunctioning SCs. These ideas led to the theory that some adult SCs suffering mutations acquire the characteristic of high cell proliferation and gain the ability to form tumours. Supporting this idea is the observation that embryonic SCs result in teratomas when implanted in vivo.

The model in which a somatic SC becomes tumorigenic is called the cancer stem cell (CSC) model. The term CSC has become more popular recently, but in the literature these cells were previously called tumour initiating cells. When isolated, CSCs have the capacity to form a new tumour in immunosuppressed mice with the same phenotype of the original tumour, supporting the differentiation of more mature progeny. Thus, the tumours are constituted with CSC populations and mature cells. The tumour has a hierarchical developmental structure in which only a fraction of the cells can proliferate indefinitely and others only act as support. Although CSCs and SCs share many features, the cancer cells exhibit genomic instability, which permits subversion of cell control and regulation.

Both types of cells exhibit self-renew capacity, and at each cell division one

*Corresponding author
Email: spbydlow@usp.br

1 Laboratory of Genetics and Molecular Hematology (LIM31), University of São Paulo School of Medicine, São Paulo, Brazil

Licensee OAPL (UK) 2014. Creative Commons Attribution License (CC-BY)

or both daughter cells retain the same biological properties as the parent cell. If the division is asymmetric, one cell retains the same properties and the other cell differentiates into mature cells, but in cancer cells the differentiation is not regulated. The attribute of self-renewal is especially notable because its potency is highly relevant to oncogenesis and malignancy\textsuperscript{15}. Despite the rarity of CSCs, some have been isolated from a number of human tumours, including hematopoietic, colon, breast, brain, and pancreatic tumours.

Unlike SCs, which are characterized by cell surface marker expression, gene expression, and tissue-specific differentiation capacities, CSCs characterization is still controversial\textsuperscript{15}.

Regarding tumour heterogeneity, the cells that originate the tumour are highly difficult to identify by specific markers. Some characteristics are currently known that allow the isolation of CSCs using cell surface markers like CD34, CD44, CD133, and CD90. In addition, the aldehyde dehydrogenase activity, the exclusion of Hoechst 33342 (for side populations that express drug transporters), or the capacity to form spheroids (when culturing in serum-free media) can be detected. The gold standard is the determination of tumourigenic capacity in immune-deficient mice\textsuperscript{16}.

These characteristics taken together can help to identify CSC and differentiate from tumours with a somatic origin. Follow we discuss some CSC recently characterized in different tissues.

Discussion

Haematological CSCs

The most well characterized tissue-specific stem cells are hematopoietic stem cells (HSCs). The modern concept of human cancer stem cells is based on studies of acute myelogenous leukaemia (AML)\textsuperscript{17}. A malignant SC population has been identified in both acute and chronic forms of myeloid leukaemia (AML and CML, respectively), as well as some forms of acute lymphoid leukaemia (ALL), using a variety of techniques, including cytogenetics, fluorescence in situ hybridization, and expression profiling by RNA analysis (reverse transcriptase-PCR)\textsuperscript{17}.

Leukaemic stem cells (LSCs) appear to retain many characteristics of normal HSCs\textsuperscript{18}. This observation indicates that the malignant SC population can arise in two possible ways. First, normal HSCs may be the direct target of mutations that cause conversion to an LSC phenotype. Second, more differentiated cell types may acquire mutations that confer SC-like properties on cells that typically would not display SC characteristics\textsuperscript{19}.

Although no universal immunophenotypic marker(s) exist that will identify all CSCs, the CD34+/CD38− population in AML has been shown to be able to transfer the leukaemia to immune-compromised mice, similar to normal HSCs\textsuperscript{20}. Primitive human LSC populations can be selected by cell surface markers CD34+/CD38−/CD123+. These cells are almost entirely quiescent, mimicking normal SCs. As a result, cell cycle agents that are active in dividing cells will not be effective with this population.

Similar to normal HSCs, a key requirement of LSCs is the ability to self-renew in order to propagate leukaemic disease. LSCs abnormally activate self-renewal pathways and anti-apoptotic signals and possess metastatic features consistent with a loss in normal SC homeostasis\textsuperscript{21}. By their nature, CSCs are biologically distinct from other cancer cell types\textsuperscript{21}. The leukaemia-initiating cells appeared to be resistant to chemotherapy and have since been blamed for the nearly unavoidable recurrence of AML after treatment that initially seems successful in eradicating the disease.

Licensee OAPl (UK) 2014. Creative Commons Attribution License (CC-BY)

Following chemotherapy, the SC-like nature of these cells enables them to replenish the leukaemic cell population, rapidly leading to recurrence of the disease. From this perspective, the cells are regarded as LSCs.

**CSCs in solid tumours**

Cancer stem cells have been isolated from several solid tumours, including colon, brain, breast, and pancreas, but not all CSCs are equal; some markers are common to all but others are different. Given the lack of prior knowledge, research is isolating cells from human tumours and identifying CSCs using a combination of some of the methods discussed above to prove the existence of CSCs in solid tumours. The search for CSCs is to understand the mechanisms underlying cancer relapse and improve treatments to overcome the several resistance mechanisms found in cancer after chemotherapy. Cells with tumourigenic capacity have only been found, isolated, and characterized in a few cancers.

Neural CSCs have been isolated by the property of growth on non-adherent surfaces, forming spheres called neurospheres. When these neuro-spheres are split into single cells, some cells can reconstitute the neuro-spheres and exhibit self-renewal potential. More recently, these cells were characterized by expression of the surface marker CD133 and form tumours when injected into mice.22,23 Other amply characterized CSCs are breast CSCs. Al-Hajj24 first demonstrated that CD24-positive and CD24-negative cells isolated from human metastatic breast cancer have the capacity to establish xenograft tumours in immunodeficient mice. This work demonstrated that only a sub-group of cells can form tumours in vivo and differentiate into all the phenotypes found in the original tumour.24

Colorectal CSCs were characterized recently using CD133 expression. Testing their tumourigenicity in vivo, these cells constituted 2.5% of the tumoural mass. When sorted, the CD133+ cell can form new tumours in mice, but CD133- cells do not.25 Similar to neural tumours, breast and colon cancer cells can grow in spheres on non-adherent surfaces.

CSCs from the pancreas were identified by engraftment assay in NOD/SCID mice. Samples of pancreatic adenocarcinoma were isolated and injected into mice; from the new tumour growth, cells were sorted for CD44, CD24, and epithelial-specific antigen (ESA) and injected into mice. Only CD44+, CD24+, and ESA+ cells were able to form a tumour with the phenotype and heterogeneity observed in the primary tumour.29

**Detracting stem cell theory**

Although many scientists accept the CSC theory for the origin of cancer, many others are against it. The CSC surface marker varies between cancer types and the results are difficult to reproduce from one cancer cell to another. The final characterization of CSCs is based in tumourigenicity in mice because only CSCs are thought to be capable of initiating tumour formation with a low number of tumourigenic cells. However, some studies have shown that the low efficiency of cancer cells at producing tumours in immunodeficient mice is a consequence of the non-human milieu, which can inhibit some cells from growing, uncovering the limitations of the assays. Therefore, the assumption that only a few cells are able to produce tumours in immunocompromised mice may actually be wrong.27

Some modifications of the xenograph assays use supports, such as Matrigel®, to induce tumour formation and some groups have shown that a few cells are sufficient to induce tumours.28,29

**Perspectives**

Further characterization of CSCs may lead to improved diagnostics and therapies. The CSC model allows us to better understand how cancer is initiated, differentiates, and in some cases metastasizes. Finding specific markers will make it possible to determine a better line of action for treatment to kill tumour cells and prevent metastases. Similarly, we could reformulate the treatment to specifically attack CSCs, avoiding or decreasing cancer relapse. On the other hand, various SCs, including HSCs, characteristically express drug-resistance proteins (such as MDR1 and other ABC transporters), which may make them less sensitive to chemotherapy drugs. CSCs can retain this phenotype and become resistant to treatment. For this reason, screens designed to identify agents that efficiently kill CSCs are needed, as they may lead to more effective cancer treatments.

**Conclusion**

Finally, if stem cells are not the origin of CSCs, the frequency with which non-tumourigenic cancer cells (those with limited proliferative potential) may evolve to acquire the properties of CSCs will need to be determined. This change may occur more readily in some cancer types than others depending on the tissue involved and could confound the effectiveness of agents that efficiently target CSCs.

**Acknowledgement**

SPB was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil; and Instituto Nacional de Ciencia y Tecnología – Fluidos Complexos, Brazil.

**References**

5. Pontikoglou C, Deschaseaux F, Senebè L, Papadaki HA. Bone Marrow