Review

Cancer stem cell hypotheses: Impact on modern molecular physiology and pharmacology research

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Although questioned on several occasions, the existence of cancer stem cells (CSCs) has been confirmed by a number of studies on experimental animal models. Nevertheless, it was shown that CSC hypotheses have several limitations and inconsistencies regarding the explanation of CSC origin, CSC identification and isolation, possible heterogeneity within CSC population, as well as methodology issues in some studies that were carried out in order to prove CSC existence. The aim of this article is to give a short and comprehensive review of recent advances concerning CSC hypothesis and to describe its impact on modern molecular physiology and pharmacology research.

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1. Introduction

Today, despite significant advances in molecular biology and clinical medicine, cancer still remains a major public health issue. Standard chemotherapy is often ineffective, and unable to eliminate all malignant cells in the body. During the past century, there have been many efforts to develop a theory that could successfully explain cancer pathogenesis and resistance to medications. In 1937, the research of Jacob Furth and Morton Kahn indicated that a single leukaemic cell, after being transplanted into a mouse, was able to produce a new hematopoietic malignancy (Furth and Kahn 1937). This led to the conclusion that within cancer cell population, certain cells might possess the ability to repopulate the cancer tissue and initiate new tumour growth. In the early 1960s, Ernest McCulloch, James Till and Andy Becker demonstrated the existence of self-renewing stem cells after injecting bone marrow into irradiated mice (McCulloch and Till 1960; Becker *et al.* 1963; Till *et al.* 1964). These and other experiments set the basis for the so-called cancer stem cell (CSC) hypotheses. The first exact proof of CSC existence was presented in 1994 by John Dick *et al.*, who successfully identified and purified human acute myeloid leukaemia-initiating cells with distinct stem properties (Lapidot *et al.* 1994).

Today, CSCs are thought to be a small population of cancer cells that have the ability of unlimited growth, self-renewal, as well as differentiation into other, more specialized cancer cell types (Ichim and Wells 2006). When transferred to immunodeficient animal models, CSCs may form new tumour tissue, whereas other cancer cells in most cases do not possess that ability. It was suggested that CSCs are able to survive and regenerate the tumour even after a large percentage of malignant tissue has been destroyed by chemotherapy.

CSC hypothesis is often regarded as an alternative to standard model of clonal evolution (CE). This model states that tumours develop as a consequence of accumulated mutations and selection of clones over period of time, resulting in a heterogeneous group of malignant cell in which cells of the dominant population (selected and expanded because of their growth advantage) have more or less equal ability for tumour regeneration and repopulation (Nowell 1976; Visvader and Lindeman 2008). However,

Keywords. CD133; CD34; Hedgehog; Notch; Wnt

Abbreviations used: ALDH, aldehyde dehydrogenase; AML, acute myeloid leukaemia; CE, clonal evolution; CSC, cancer stem cell; IL8, interleukin 8; LIF, leukaemia inhibitory factor; PTL, parthenolide; SCID, severe combined immune-deficient

although questioned on several occasions, the existence of CSCs has been confirmed by a number of studies on experimental animal models. CSCs have been identified and isolated in various hematological as well as solid malignancies. In recent years, various research strategies have been focused on detecting specific surface markers and signalling pathways that could in the future lead to development of medicaments capable of targeting CSCs.

On the other hand, it was shown that CSC hypotheses has several limitations and inconsistencies regarding the explanation of CSC origin, CSC identification and isolation, possible heterogenicity within CSC population, as well as methodology issues in some studies that were carried out in order to prove CSC existence. Therefore, many authors today suggest that neither the CSC model nor the CE model should be ruled out when explaining cancer pathogenesis, and that the answer lies in the combination of these two models.

The aim of this article is to give a short and comprehensive review of recent advances concerning CSC hypothesis and to describe its impact on modern molecular physiology and pharmacology research.

2. Cancer stem cells in hematological and solid malignancies: Evidence of CSCs existence and identification of CSC markers

One of the first studies that significantly contributed to formation of CSC hypotheses was published in 1994 (Lapidot *et al.* 1994). The authors identified a subtype of acute myeloid Leukaemia (AML) cells with specific surface marker phenotype (CD34+ CD38−) that were able to produce large numbers of colony-forming progenitors when transplanted into severe combined immune-deficient (SCID) mice. CD34+ CD38+ and CD34− cells did not have these properties. These experiments confirmed the earlier line of thought that only a fraction of malignant cells has the capability to generate and sustain new tumour tissue upon transplantation. In 1997, Bonnet *et al.* also demonstrated that the cell capable of initiating human AML in SCID mice possesses the differentiative and proliferative capacities and the potential for self-renewal expected of a leukemic stem cell (Bonnet and Dick 1997).

Today, it is known that CSCs are present in various solid tumours as well. Breast CSCs were first identified as $CD44^{+}CD24^{-/low}$ cells in samples of human breast tumours (Al-Hajj *et al.* 2003). According to these authors, only 100 cells with this phenotype were enough to form tumours in SCID mice, whereas tens of thousands of cells with alternate phenotypes failed to form cancer tissue (Al-Hajj *et al.* 2003). A recent study on breast cancer cell lines confirmed the presence of functional CSCs with metastatic capacity and a distinct molecular signature (Charafe-Jauffret *et al.* 2009). Some breast CSCs were identified by detecting the expression of a specific stem cell marker, aldehyde dehydrogenase (ALDH), using fluorescent ALDEFLUOR assay as previously described (Ginestier C *et al.* 2007). After quantitative RT-PCR assessment of CSC population, it was shown that interleukin 8 (IL8) receptor CXCR1/IL8RA was consistently expressed in the ALDEFLUOR-positive cell population, indicating that the IL8 signalling pathway may be of great importance for CSC function (Charafe-Jauffret *et al.* 2009). Also, breast CSCs are thought to be stimulated by estrogen through the FGF/Tbx3 signalling pathway that similarly controls normal mammary epithelial stem cell biology (Fillmore *et al.* 2010).

There is a considerable amount of evidence that brain cancers are also hierarchically organized. Human medulloblastoma CD 133+ cells were shown to be capable of initiating new tumour tissue (consisting of both CD 133+ and CD 133− cells) after intracranial transplantation into SCID mice (Singh *et al.* 2004). There are also reports that glioma stem cells have some unique properties, such as preferential activation of the DNA damage response, which makes them resistant to radiotherapy (Bao *et al.* 2006). Those reports suggest that CD133+ tumour cells could be the source of tumour recurrence after radiation (Bao *et al.* 2006). The exact signalling pathways that regulate brain CSC function (self-renewal, differentiation, etc.) are largely unknown. Two years ago, it was suggested that TGF-beta can induce the self-renewal capacity of glioma stem cells probably via the Smad-dependent induction of leukaemia inhibitory factor (LIF) and the subsequent activation of the JAK-STAT pathway (Peñuelas *et al.* 2009). Also, there are reports that the 'stemness' (stem-like properties) of brain CSCs may depend on T-cell activity (Irvin *et al.* 2010). We assume that in the next 5 years significant research efforts will be focused on clarifying the relationship between CSCs and immune responses modulation.

The existence of tumorigenic and non-tumorigenic cells within colon cancers has been shown by a number of recent studies (O'Brien *et al.* 2007; Ricci-Vitiani *et al.* 2007). All experiments were performed in immunodeficient mice, and CSCs were shown to be CD133-positive. Several research steps have been taken in order to identify a specific signalling pathway that could eventually lead to growth inhibition and destruction of these cells (de Sousa *et al.* 2011).

Recently, it has been suggested that human melanomas also contain tumour-initiating cells (Schatton *et al.* 2008). Melanoma CSCs were defined by expression of the chemoresistance mediator ABCB5. These authors stated that specific targeting of this tumorigenic minority population could inhibit tumour growth and impact the prognosis of the disease (Schatton *et al.* 2008). CD133 and ABCG2 molecules are also associated with melanoma stem cells

(La Porta 2009). Still, many issues concerning these markers in melanoma remain unclear. Although CD133 and ABCG2 are sometimes expressed in cells with proportionately higher tumour propagating activity than other malignant cells, a clear demonstration of their involvement in self-renewal capacity in melanoma cancer stem cells still does not exist (La Porta 2009).

All in all, we can conclude that, so far, there is strong evidence that CSCs are present in a majority of solid and hematological malignancies that affect human population. Detection of specific surface markers that characterize CSCs is an important step that may impact future strategies in anti-cancer drugs design and development. Nevertheless, further research efforts are required determine the clinical significance of these findings, and to show whether CSCs are universal property of all cancers.

3. Cancer stem cell hypotheses has several limitations and inconsistencies

When the concept of CSCs was first formulated, many researchers thought that the standard model of CE of cancer needed major revision, and that CSC hypotheses could eventually even replace it as one of the main principles of cancer pathophysiology. However, several questions about CSCs function and role in cancer tissue remained unanswered.

First of all, there are issues concerning the methodology used in the studies to identify CSCs. Most of the *in vivo* experiments have so far been done on animal models (immune-deficient mice) injected with cells obtained from human cancer tissue samples. It is not clear how this xenotransplantation may affect the validity of the obtained data. These issues were addressed in the study by Kelly *et al.*, who isolated primary pre-B/B lymphoma cells from three independent Eμ-*myc* transgenic mice and injected 10 to 10^5 cells into non-irradiated congenic animals (Kelly *et al.* 2007). The results of the experiment showed that regardless of the cell number injected, all animals developed disseminated lymphoma within 35 days, suggesting that the concept of cancer growth being always sustained by a rare cancer stem cell may be wrong (Kelly *et al.* 2007). Indeed, the main question that we should ask from this congenic transplantation study is, why are so few cells enough for cancer initiation when CSCs are thought to represent only a small fraction of tumour cell population? However, in a response to Kelly's work, Kennedy *et al.* indicated that the use of xenotransplantation model is justified, having in mind the development of new, more sensitive xenograft assays that include depletion of residual immune activity, direct injection of cells into bone cavities and transgenic expression of human cytokines (Kennedy *et al.* 2007). Although these improved assays were able to detect higher percentages of CSCs, the preliminary data suggested that CSCs were still relatively rare in cancer cell population. For example, a novel genetically induced model of human B cell acute lymphoblastic leukaemia (B-ALL) transplanted into in immunodeficient mice showed the frequencies of leukaemia-initiating cells among leukemic blasts of approximately 1% (Barabé *et al.* 2007; Kennedy *et al.* 2007). We assume that although xenotransplantation models have certain limitations, especially concerning the effects of murine microenvironment on cancer cell behaviour, for the time being, xeno-transplantation will remain a model of first choice for *in vivo* research, since mouse-to-mouse transfer may require a lot of financial resources and is difficult to carry out.

Secondly, it is speculated that the CSC population itself may in some circumstances show some degree of heterogeneity. For example, recently it was demonstrated that Brca1-deficient mouse mammary cancers contain heterogeneous cell populations (CD44+/CD24− and CD133+) with CSC characteristics (Wright *et al.* 2008). In acute myelogenous leukaemia, it has been reported that stem cells are rare and heterogeneous when assayed in SCID mice (Sarry *et al.* 2011). The authors of this study suggested that CSC phenotype possesses a certain degree of plasticity which was not reported in previous

Table 1. Important markers associated with cancer stem cell research

Important molecular markers associated with cancer stem cell research	Tumour type	References
CD34	Leukaemia	Lapidot *et al.* 1994
CD44	Breast	Al-Hajj *et al.* 2003
CD 133	Brain, colon, breast, melanoma	Singh *et al.* 2004; Bao *et al.* 2006; Ricci-Vitiani *et al.* 2007; O'Brien *et al.* 2007
ABCB5	Melanoma	Schatton *et al.* 2008
ALDH1	Breast	Ginestier *et al.* 2007
$CD24^{-/low}$	Breast	Al-Hajj *et al.* 2003

investigations (Sarry *et al.* 2011). There are several possible explanations for CSC heterogeneity. According to one them, some cancer tissues contain cancer progenitor cells that are more differentiated than CSCs but still possess some residual stem cell traits (Fábián *et al.* 2009).

Also, we should always have in mind that there is a possibility that classical stem cell hierarchy may not be present in all tumours. Some researchers speculate that populations of non-CSCs from various types of tumours have greatly differing susceptibilities to becoming CSCs in response to specific signals (Gupta *et al.* 2009). This 'interconversion' between the two populations, if proven, threatens to relativize the whole CSC concept. According to some other views, CSC assay conditions might have a significant effect on the success rate of transplantation, and thus, it may turn out that CSCs are not as rare as previously thought, particularly having in mind that much depends on recipient microenvironmental/niche factors (Bomken *et al.* 2010). Having in mind all limitations of CSC hypotheses, many authors agree that a more complex approach that includes both CSC and CE models are required for better understanding of cancer pathophysiology.

4. Future of CSC research

We have reason to believe that in the next decade, main research efforts concerning CSCs will be focused on developing new *in vivo* and *in vitro* methods suitable for identification of CSCs and their markers in hematological and solid malignancies (table 1). The very existence of CSCs has been proved on many occasions; however, the present models of cell organization and interaction in tumour tissue will have to be revised if we are to understand the main principles of tumour growth and metastasis. If CSCs are shown to be main (or only) cause of tumour propagation, the primary goal of our research should be to develop a medicament that could specifically target and destroy CSCs, overcoming their documented ability to develop drug resistance. To achieve this goal, various signalling pathways in CSCs will need to be identified and described. Several important steps in this direction have already been made by a number of laboratories. It is, for example, known that standard pathways for self-renewal of normal stem cells, such as Wnt, Notch and Hedgehog signaling, are also present in CSCs and have an important role in their function. Targeting critical steps in those pathways, however, will be complicated by signalling cross-talk as well as other issues (Takebe *et al.* 2011). Nevertheless, there are already several reports that CSCs can be selectively targeted without inflicting serious damage to normal stem cells (Visvader and Lindeman 2008). For example, parthenolide (PTL), an active component in Feverfew (*Tanacetum parthenium*), was found to selectively induce programmed cell death (apoptosis) in leukaemic stem cells, possibly by activation of proapoptotic p53 signalling pathway (Guzman *et al.* 2005). Novel types of monoclonal antibodies directed to the adhesion molecule CD44 can also specifically target leukemic stem cells (Jin *et al.* 2006). Finally, in some solid tumours, such as gliomas, a combination of targeted antiangiogenic therapy and cytotoxic chemotherapy can selectively reduce the CSC fraction (Folkins *et al.* 2007). These and other findings give us hope that in the future, novel, more efficient and less toxic anti-cancer medications could be developed. However, to achieve this objective, a multidisciplinary approach to CSC issue is required, including the cooperation between molecular medicine researchers and clinical practitioners.

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