www.cicancer.org

How to Eliminate Cancer Stem Cells

CSIC
Consejo Superior de Investigaciones Científicas

cic
Centro de Investigación del Cáncer

Our partners

CSIC
Consejo Superior de Investigaciones Científicas

CIC
Centro de Investigación del Cáncer

IBSAL
Instituto de Investigación Biomédica de Salamanca

Universidad de Salamanca

Dr Isidro Sánchez-García is a scientist working in the Institute of Molecular and Cell Biology of Cancer at the CSIC/University of Salamanca, Spain, to debilitate cancer. While his research has focused on different haematological cancers, his work into targeting malignant stem cell populations to eradicate cancerous tumours may have a major impact on the concepts, therapies and methods for assessing treatment efficacy of cancer biology and development across the board.

INTRODUCTION

Cancer is the second cause of death in developed countries, and its incidence is steadily on the rise. In general, since 1930, cancer death rates have increased with age for both males and females, and the age-specific occurrence of cancer was similar for both genders. Furthermore, the 5-year-mortality trends for the most relevant types of tumors have only slightly receded (only up to a 3.3% for prostate cancers) or even have increased (2.2% for liver cancer) in the last 40 years (Marshall, 2011; Wingo et al., 2003), in spite of the enormous amounts of money invested in cancer research during this period. Most of the advances in the field of cancer treatment have been related to the early detection programs that have increased our chances of identifying cancers in very early stages (Etzioni et al., 2003). However, given the disseminated nature of the disease (Husemann et al., 2008; Sanchez-Garcia, 2009), in many cases this early detection comes already too late, and the prognosis for metastatic cancers is today as dark as it was 50 years ago.

Current therapeutic approaches treat cancer as a disease of proliferation, and pay little or no attention to the internal structure of the tumor or its cellular heterogeneity. However, we now know that cancers are much more than uncontrolled cell lines, and that there is an internal hierarchical structure within the tumor, with cells with different biological properties and different sensitivities to therapy. This chapter is based on the assumption of the main postulates of the cancer stem cell (CSC) theory. A full discussion of the CSC concept and its implications is therefore out of the scope of the chapter and, for this, the reader is referred to other chapters in this book and to other recent reviews. We will, however, discuss, in the first part of this review, the most recent results that are finally bringing CSCs to the forefront of the study of human cancer, and that are starting to show the implications of CSCs in prognosis, malignancy and disease evolution in humans. Afterwards we will revise the most recent discoveries in the research aimed at the targeting and elimination of CSCs.

THE COMING OF AGE OF CANCER STEM CELLS

At the basis of the CSC theory is the concept of cancer as a stem cell-based, hierarchically organised, aberrant tissue, in which only a subset of cells (the CSCs) have the capacity of maintaining and re-generating the tumor. In current anti-cancer therapies, most drugs are aimed at interfering, by several mechanisms, with the replication of the rapidly-dividing cells that conform the majority of the tumoral mass. This approach, which causes many highly undesirable side-effects because of its lack of specificity, is able to eliminate the clinically visible tumor mass ("cancer cure") in many patients. However with the current detection techniques, it is impossible to detect tumoral cells when their number is below 109. Therefore, "cancer cure" refers to a state in which tumoral cells are below this detection limit… but there is a wide range between 109 and 0 tumoral cells, as it is clear from the high frequency of relapses in patients that had initially achieved clinical remission after chemo- or radiotherapy cycles. The high frequency of relapses indicates that the cells responsible for tumor replenishment are not being affected by the conventional therapy and, with time, they regenerate the tumor. It is true that there are examples of successful treatment of certain tumor types with current therapeutic regimes, like the high rate of cure (80%) of most types of childhood acute lymphoblastic leukemias. However, even in these cases, the use of highly toxic, unspecific drugs comes at a price, since most patients develop treatment-related diseases (including cancers) in their adulthood.

It is therefore clear that a new conceptual framework is required in our approach to treat cancer. Cancer is a disease of genetic origin, and men and women in developed countries have a 0.5 and a 0.33 lifetime risk of developing cancer, respectively. This high incidence is possibly an indication of the fact that cancer is not just "an unfortunate accident", but rather a catastrophe waiting to happen, a latent possibility intrinsic to our cells, as the cost we have to pay for being a complex multi-cellular organism with many different developmental pathways controlled with "just" one genome. From this point of view, cancer is an aberrant differentiation program, established as a consequence of one or a series of oncogenic alterations that open the way for a new pathologic lineage to appear and develop. This perspective of tumorigenesis moves the focus away from proliferation and towards the alteration of the normal differentiation programs: cancer is a disease of aberrant reprogrammed differentiation, and as such must be studied and (hopefully) treated. Normal tissue development is a consequence of stem cell programming and stepwise commitment towards specific lineages. Cancer development is a consequence of the tumoral reprogramming that arises from the interaction between the driving oncogenic events and the plasticity of the cancer cell-of-origin where these events initially take place.

All these aforementioned concepts, that were explicitly or implicitly laid down by the pioneers many years ago, have been thoroughly explored by many research groups in the 2000's decade, mainly by using xenografts of prospectively purified human tumoral cells into immunodeficient mice, or by using genetically engineered mouse models of cancer. Once this groundwork has being laid, in the last few years special stress has been put in demonstrating that all these biological findings are indeed of some clinical relevance to humans. Certainly one would expect that, if CSCs are essential for tumor survival, then the characteristics of these CSCs should have some impact in the tumor biology. That this is indeed the case has recently been demonstrated for several types of tumors.

In spite of the previously described conclusions, caution is always required when interpreting results in a research field as new and quickly evolving as this one. In most of the reports it is assumed that the association of a CSC signature with a bad prognosis is directly related to the higher number of CSCs present in the corresponding malignant tissue. However, in general, CSCs constitute a very reduced percentage of the tumor, therefore making it unlikely that they can significantly contribute in a relevant manner to a global tumoral gene expression profile. Thus, summarising the relevance of these last years' findings, in spite of all the caveats, they clearly have

important biological and clinical implications regarding CSCs and their role in tumor biology. First, the clear relationship between the CSCs' and other stem cells' signatures, and the fact that these signatures indeed seem to predict survival, provides evidence for the hierarchical organization of cancers according to the CSC model and confirms that CSCs are not experimental artifacts. Moreover, CSCs, defined on the basis of functional stem cell properties, are distinct from other, non-CSC tumor cells, and are clinically relevant. Therefore, it is highly likely that therapies targeting CSCs would improve patient survival, and that animal models based on the CSC theory would be useful in the preclinical evaluation of new cancer drugs. Finally, the identification of transcriptional profiles conserved between CSCs and other types of stem cells suggests that there are specific genes in charge of establishing and maintaining the stem cell state, and that they might influence clinical outcome.

KILLING CANCER STEM CELLS: AIMING AT A MOVING TARGET

It is clear therefore that CSCs are relevant therapeutic targets if we want to achieve definitive remissions in cancer patients. The key question is then: how do we target them? There are two main aspects to this problem: i) which cells are the real CSCs? and ii) what are the specific molecular targets that we need to attack – to kill them or to interfere with their function without affecting normal stem cells?

Regarding this first question (which cells are the real CSCs?), until now the search for CSCs has been based on the prospective purification of cancer cell subpopulations and the determination of their cancer-transplantation capacities into immunodeficient mice. This approach, being very powerful, presents nevertheless several methodological shortcomings that have been described in detail elsewhere. However, beyond the technical deficiencies, the biology of CSCs themselves poses many difficulties for their identification. One of the most relevant problems is the elusive nature of the CSCs. Already at the earliest stages of the tumor, the path from the cancer cell-of-origin (CCO, the cell suffering the first genetic lesion associated to cancer development) to the CSC is complex, and impossible to determine in human cancers, which are diagnosed when they have already evolved into full-blown tumors. Traditionally, the cellular origin of tumors was extrapolated to the most phenotypically similar normal cells. However, if tumors are stem cell-based tissues, clearly this association cannot be made, and the search for CSCs is providing many examples of this fact.

Beyond the blurred origin of the CSCs, their 'mature' nature is not less complicated. In childhood B-cell acute lymphoblastic leukemias, it has been shown that CSC-activity can be identified in blasts at different maturational stages, therefore making it difficult to identify a single cellular component as the one responsible for tumor maintenance. In this type of hematopoietic tumor it has been shown that the leukemia-propagating cells are genetically variegated, presenting subclonal patterns with different competitive regenerative capacities *in vivo*. Genetic variegation of CSCs may represent a great block to effective treatment if the molecular targets identified are not the cancer-initiating lesions, but rather secondary mutations segregated in subclones, even when these ones finally appear as the dominant ones. Finally, and to complicate the problem even more, it is increasingly clear that there is an evolution of the nature of CSCs during the progression of the disease, including the response to treatment and final relapse, indicating that CSCs are capable of evolving ways of survival in response to the changes in the environment and other external selective pressures.

All these data evidence the tremendous difficulty of finding the right cellular targets at the time of treatment (diagnosis, relapse, etc), and how much we still need to learn about the biology of cancer stem cells in order to be able to attack them. The current research in this field is tremendously active and it builds to a large degree (for both good and bad) on our previous experience in the

study of cancer cells in general. Still, as we have explained before, one essential change introduced by the acceptance of the CSC theory has been to move the focus of the research in cancer therapies from proliferation to differentiation and stem cell biology. Therefore, the putative CSC-specific targets are being mainly sought, in a prospective manner, among the most relevant of the molecular signaling routes involved in the regulation of fate determination and in the specification and maintenance of the stem cell identity. These signaling routes, together with some other approaches of less relevance (due to their more restricted applicability at the moment) like differentiation therapies, constitute what we could already call the "classical" anti-CSC targets. On the other side, due to our general lack of knowledge about the biology of stem cells, research in this field is also revealing new unexpected potential anti-CSC targets related with a wide variety of cellular processes.

FUTURE PROBLEMS, FUTURE PROSPECTS

Now we have reviewed the most relevant results from the scientific literature in the last 3-4 years and, although several new potential anti-CSC targets have been identified, the aftertaste is still somewhat sour: no real breakthroughs have been made. Furthermore, research in the field of anti-CSC-targeted therapy is starting to look disappointingly similar to the basic cancer research of the last 20 years: many small incremental findings about minor details of molecular mechanisms, usually in very specific experimental contexts. All very interesting and new, but all of it too far from any realistic therapeutic application. If we really want the CSC theory to make a difference on the way we treat cancer, we really have to start accepting all the implications of the theory, rather than keeping on doing the same kind of research and just adding the "CSC" label to it.

From our point of view, new research tools and approaches are required that take the CSC hypothesis into account from a bottom-up perspective. This means that the CSC theory should be the starting point for the experimental design, rather than the top-bottom approaches used today, in which old systems and models are still used, just pretending that they are equally useful under the constrains of the CSC theory as they were before. All these screenings must, for obvious throughput reasons, be performed *in vitro*. However, their *in vivo* validation in a suitable model is an essential requisite to move them forward into trials. Indeed, we have mentioned some of the problems related to the need of avoiding toxicity to normal stem cells. But these are not the only ones, there can be many other unexpected difficulties. For example, stem cells might not be the only important cells requiring a particular molecular route. Indeed, there are other types of cells that can present stem cell-like features at certain points of their life, and therefore an unspecific therapy might have unwanted effects on them. For instance, in the mouse immune system, both memory T and memory B cells share a transcriptional self-renewal profile with HSCs, a property that has also been found in humans, and B cells in the germinal center have been shown to divide asymmetrically, a property highly related to stem cell self-renewal. Another problem might be related from the fact that maybe more than one type of stem cells contribute to generate a given tissue, so testing *in vitro* the toxicity against a certain stem cell type might not account for the full effect that a drug might have *in vivo* on tissue renewal. Therefore, the development of *in vivo* models that can accurately reproduce CSC-driven tumors similarly as how they happen in humans is not just an essential step for our understanding of the biology of CSCs and for the identification of new anti-CSCs targets and biomarkers but, furthermore, improved tumor models will be required as the definitive system in which new therapies can be tested before they can be translated into humans. Since, as of today, most models are still based in theoretical concepts that ignore the CSC theory, the generation of CSC-based animal models will be the main bottleneck in the coming years for the discovery, development and translation of new real anti-CSC targets that can help the patients.

www.cicancer.org

Institute of Molecular and Cell Biology of Cancer
Campus Miguel de Unamuno
37007 - Salamanca (Spain)
Tel: 34 923 294 720
Fax: 34 923 294 743
e-mail: cicancer@usal.es

Our funding partners

Arimmora
GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN
Junta de Castilla y León
NIH National Institutes of Health
DECIDE
PEOPLE