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Extra View

Stem-cell driven cancer

"Hands-off" regulation of cancer development

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A cancer dogma states that inactivation of oncogene(s) can cause cancer remission, implying that oncogenes are the Achilles' heel of cancers. This current "hands on" model of cancer has kept oncogenes firmly in focus as therapeutic targets and is in agreement with the fact that in human cancers all cancerous cells, with independence of the cellular heterogeneity existing within the tumor, carry the same oncogenic genetic lesions. This rule has now been broken in a study of the effect of the *BCR-ABL* oncogene in cancer development in a mouse model in which oncogene expression is restricted to the stem cell compartment. *BCR-ABL* is linked to chronic myeloid leukemia (CML) disease in humans, and this study shows that by limiting the oncogene expression to Sca1$^+$ cells CML arises, indicating that maintenance of oncogene expression is not critical for the generation of differentiated tumor cells and showing a "hands off" role for *BCR-ABL* in regulating cancer formation. Here we provide an update on the use of this system for modeling human cancer and its potential application for therapeutic targeting of cancer stem cells (CSCs) and the hands-off function of oncogenes.

Introduction

Since the discovery that human tumors contain activated oncogenes by the pioneer work of Mariano Barbacid, Geoffrey Cooper, Robert Weinberg and Michael Wigler,[1-4] many efforts have been made to elucidate the causal role that these oncogenes play in cancer development. These previous works have shown that oncogene expression is not only required for initiation of cancer but also for the maintenance of the disease and have kept oncogenes firmly in focus as therapeutic targets. In mouse models where oncogene expression is driven by tissue-specific promoters, tumors arise at high frequency, but disappear again when the inducing stimulus is switched off,[5-7] suggesting that oncogenes are the Achilles' heel of cancers.[8] Overall these observations define an homogenous role for oncogenes within cancer cells, as brief inactivation of the single tumor-inducing oncogene can cause remission in these model systems. These observations are consistent with a "hands on" role for oncogenes in regulating tumor formation in a similar way to the control of cell-fate determination as a function of lineage-specific factors.[9]

This current "hands on" model of cancer is in agreement with the fact that in human cancers, all cancerous cells carry the same oncogenic genetic lesions. However, it is also a very well-known fact that cancers are composed by heterogeneous cell types,[10,11] suggesting that, in the control of oncogenesis, the nature of the target cells suffering the effects of oncogenic activity might play an important role. In fact, therapy based on the "hands on" model of cancer fails to eradicate tumors in humans, as it is well illustrated by the BCR-ABL kinase inhibitors such as imatinib/STI571, which can target the differentiated tumor cells of chronic myeloid leucemia (CML) but fail to eradicate the BCR-ABL-expressing stem cells.[12,13] On the contrary, these observations are compatible with the cancer stem cell (CSC) theory of cancer that suggests that tumors are hierarchically organized tissues.[14-16] If that was indeed the case, then cancer could be created and maintained similarly to any other normal stem cell-driven tissue, like the hematopoietic system. In a normal stem cell-driven tissue, genetic programming of stem cells is all what is required to (re)constitute all differentiated cells forming the tissue, and the genetic information responsible for the stem cell programming does not need to be anymore present within the differentiated cells that form the tissue, implying a different function for oncogenes within CSCs. Thus, we reasoned that a similar organization could be underlying cancer formation. In order to initially address this biological question, we have used the *BCR-ABLp210* oncogene.[17]

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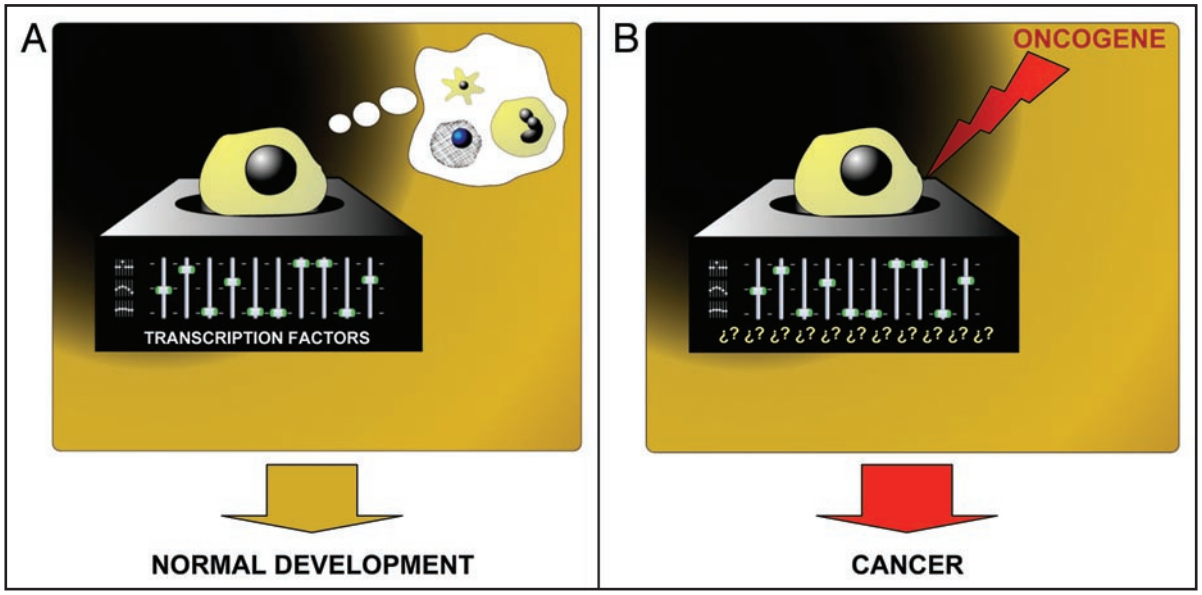

Figure 1. A "hands off" role for BCR-ABL in regulating CML formation. (A) Adopting a lineage amongst two or more options is a fundamental developmental decision in multicellular organisms. The control of cell-fate determination of a stem cell is a function of the balance between lineage-specific factors. (B) In our stem cell-driven cancer model, the expression of the oncogenic alteration is restricted to the progenitor compartment but is nevertheless capable of generating a full-blown tumor with all its differentiated cellular components, showing a hands-off role for BCR-ABL in regulating CML formation. This model implicitly relies in the fact that the oncogenic presence in the CSC compartment originates (epi)genetic latent alterations which are responsible for the posterior appearance of the tumoral phenotype.[17] Consistent with this, forced expression of these genes can—in certain cellular environments—select or impose a lineage outcome, explaining why most chimeric oncogenes created after chromosomal translocations are found only in one type of tumor.

Limiting BCR-ABLp210 Expression to Stem Cells Induces CML in Mice

A major barrier for the understanding of the contribution that CSCs make to the development and maintenance of cancer and their suitability as a target was the lack of a system to limit oncogene expression to the CSC compartment. To elucidate if cancer is a stem cell-driven tissue, we used the Sca1 locus control region to limit oncogene expression to the stem cell compartment in a transgenic mouse setting.[17] We have initially focused on the effects of the *BCR-ABLp210* oncogene, linked to chronic myeloid leukaemia (CML) in humans.[18,19] CML is widely accepted to be a stem-cell disorder that begins as a prolonged chronic phase, characterized by high leukocyte counts and enlarged spleen and liver. In all patients, the chronic phase of CML in the end gives rise to a blast crisis that is indistinguishable from acute leukemia. The specific BCR-ABL inhibitor STI571 is able to eliminate the BCR-ABL-expressing differentiated cells that constitute the bulk of the tumor, but it cannot eliminate BCR-ABL-expressing CSCs.[12,13]

When the expression of BCR-ABL is restricted to the $Sca1^+$ cells in mice, these *Sca1-BCR-ABLp210* mice fully develop CML. In these *Sca1-BCR-ABLp210* mice, although initiation takes place within the stem cell/progenitor population, the oncogene is switched off in the tumor differentiated cells which constitute the bulk of the tumor. In the paper by Perez-Caro et al.[17] quantitative RT-PCR analysis of *BCR-ABL* transcripts was used to define patterns of *BCR-ABL* gene expression in pure populations of hematopoietic cells. Overall, *BCR-ABL* is not expressed in lineage-positive hematopoietic progenitors, but it could be that BCR-ABL target genes could continue to be expressed in the absence of BCR-ABL. These genes could be targets of a "hit and run" mode of action in which BCR-ABL turns genes on in stem cells but is not required for maintaining their expression at later stages of development. However, neither BCR-ABL protein nor downstream signaling were detected in $Sca1^-Lin^+$ cells of *Sca1-BCR-ABLp210* mice. The data support the hypothesis that BCR-ABL downstream targets are switched off after the silencing of *BCR-ABL*. It might appear then counter-intuitive and surprising that cancers develop efficiently in these mice since in actual human cancers all cancerous cells carry the oncogenic genetic lesions, not only the cancer stem cells. Nevertheless, CML arises in these mice indicating that silencing of *BCR-ABL* is not critical for generation of differentiated tumor cells and suggesting a "hands off" role for BCR-ABL in regulating tumor formation (Fig. 1).

To determine whether the continuous presence of BCR-ABL is necessary for the maintenance of CSCs we treated diseased *Sca1-BCR-ABLp210* mice with the specific BCR-ABL inhibitor STI571 and we found that the course of the CML disease was not modified upon treatment. These observations demonstrate that blocking BCR-ABL function (or at least abolishing its tyrosine-kinase activity) is not efficient in eliminating the CSCs, in agreement with both of the above-mentioned observations showing that STI571 treatment fails to eradicate the *BCR-ABL*-expressing stem cells in human patients[12,13] and the development of CML in our BCR-ABL "hands-off" model (Fig. 1).

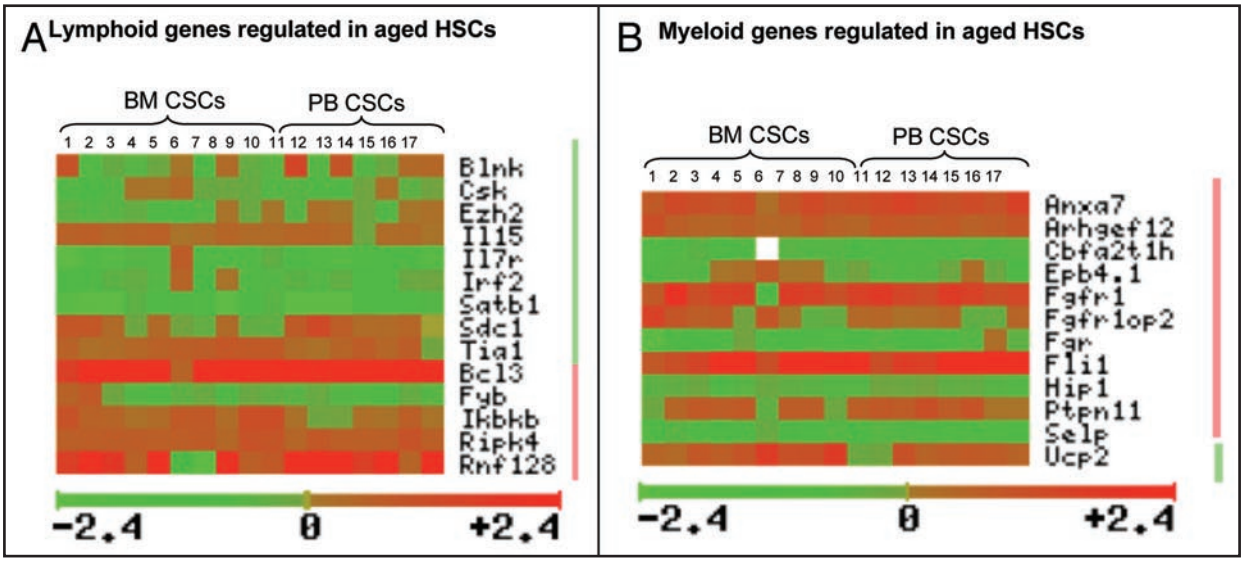

Figure 2. Cancer stem cells in *Sca1-BCR-ABLp210* mice share features with aged stem cells. To identify genes associated with *BCR-ABLp210*-induced reprogramming of stem cells, we have compared the gene expression profiles of purified CSC populations versus normal HSCs. Both CSCs and HSCs were isolated as $Sca1^{+}Lin^{-}$ cells. The figure represents a graphical description of the patterns of expression, in CSCs from *Sca1-BCR-ABLp210* mice, of lymphoid (A) or myeloid (B) genes previously identified as being differentially regulated in old HSCs.[20] Genes were selected for which signal intensities were changed ± 2 in CSCs vs. HSCs. We referred the ratios of the CSCs to the control hematopoietic stem cells ($Sca1^{+}Lin^{-}$ cells purified from control mice). Each gene (identified at right) is represented by a single row of colored boxes; each experimental mouse is represented by a single column. Data are displayed by a color code. Red fields indicate higher values than the median, green fields indicate lower values than the median. The colour bar on the right site of each panel indicates genes upregulated (red) or downregulated (green) in aged HSCs.[20]

But because we wanted to address the question of whether CSCs are required continuously for maintenance of the CML disease, we used a model in which $Sca1^{+}$, *BCR-ABL*-expressing cells are deleted in the presence of gancyclovir. After elimination of the CSC, we were able to eradicate the whole tumor. These observations formally prove that CSCs are required continuously for maintenance of the CML disease. However, abolishing BCR-ABL function is not critical for the generation of differentiated tumor cells. In our view, this constitutes the most convincing evidence to date that these cancers arise and are driven by a cell fate change within the stem cells, and that this population is the ultimate target for cancer therapy. In total, the data suggest a "hands off" role for BCR-ABL in regulating cancer formation.

How Does BCR-ABL Program Stem Cells to Make a CML?

In order to identify the genes that are associated with BCR-ABLp210-induced reprogramming of stem cells we performed a supervised analysis of the transcriptional profiles of CSCs purified from *Sca1-BCR-ABLp210* mice versus HSCs from control mice. The data identified a set of genes that are reproducibly differentially regulated in CSCs versus control stem cells. Because the CSC hypothesis could imply that cancer results from the activation of dormant embryonal-rest cells,[10,11] we next proceeded to examine, in CSCs from *Sca1-BCR-ABLp210* mice, the expression of embryonic surface markers that have been previously identified in undifferentiated mouse embryonic stem cells and we could show that CSCs in *Sca1-BCR-ABLp210* mice present embryonic figures.[17] Although overall these observations identify potential attractive targets for selective CSC removal, they may also just reflect the function of the oncogene at the stem cell level.

One possible hypothesis would be that the *BCR-ABL* oncogene activity might be promoting the aging of the targeted stem cells. In this regard, it has been described by expression profiling tools that HSC aging is accompanied by the downregulation of genes mediating lymphoid specification and function and upregulation of genes involved in specifying myeloid fate and function.[20] Thus we next examined the expression of genes associated to physiological HSC aging[20] in CSCs from *Sca1-BCR-ABLp210* mice. A shown in Figure 2, CSCs from *Sca1-BCR-ABLp210* mice share several features with aged stem cells. These data support a model in which stem cells may be affected in similar ways by aging and by oncogenes. As young stem cells are more resistant to chemotherapy than aged stem cells, these findings suggest a therapeutic window opportunity to destroy the CSCs while keeping intact the normal stem cell compartment.

A "Hands Off" Role for BCR-ABL in Regulating Tumor Formation

In human pathologies and in most animals models of cancer, the oncogenic alteration(s) is(are) present in all the cellular types that compose the tumoral tissue, from the cancer stem cells to the more differentiated types. In our stem cell-driven cancer model, the expression of the oncogenic alteration is restricted to the progenitor compartment but is nevertheless capable of generating a full-blown tumor with all its differentiated cellular components, showing a hands-off role for BCR-ABL in regulating CML formation. Of course, this model implies that the oncogenic activity in the cancer stem cell compartment causes (epi)genetic latent

alterations which are responsible for the later appearance of the tumoral phenotype.[17] In fact, our initial characterization of CSCs has shown that BCR-ABL causes secondary genetic or epigenetic changes (such as DNA methylation, centrosome abnormalities and consequent aneuploidy, and others) which may be inherited by subsequent generations regardless of the lack of continued *BCR-ABL* expression. Thus, *BCR-ABL* oncogene inactivation cannot change this epigenetic/genetic context at the CSC level, in agreement with the common occurrence of tumor relapse by which human CML evolves to escape BCR-ABL pharmacological inactivation. Moreover, these results show that the control of oncogenesis is a function of both the target-cell and the genetic oncogenic alteration(s), being the molecular mechanisms of action of BCR-ABLp210 at the CSC level different from those acting at later stages of tumoral cell differentiation.

Conclusions and Future Directions

Despite a better understanding of the biology of tumor cells, the treatment of most cancers has no significantly changed for the past four decades and the decreasing mortality has been mostly the result of early detection and prevention rather than the consequence of effective therapeutics.[21-23] Thus, the cells and genetic lesions responsible for maintaining the disease remain an intriguing and exciting topic of research, since these cells have been posited to be responsible for resistance to conventional therapies, recurrence and metastasis.[24]

We have shown that cancer growth and elimination was achieved by targeting oncogene expression to the CSCs only.[17] Thus, if the growth potential of a cancer depends on CSCs and on oncogenes that can function in a hands-off manner, it seems important to know how to eradicate these cells and/or inactivate the hands-off mechanism. Similarly, assessing the ability of any candidate therapy to destroy these cells would seem crucial to predicting its efficacy.[25] The evidence for the existence of CSCs in human tumors is based on the creation of mice that are sufficiently immunodeficient to tolerate the growth of primary human cells into them.[25] However, this growth does not exclude the possibility that engraftment rather than cancer stem cell activity correlates with a particular phenotype.[26] CSC-based models, as the one described in Perez-Caro et al.[17] allow bypassing the limitations and experimental variability of the xenotransplant models. An enormous advantage of our CSC-based models is that they not only enable syngeneic transplantation but they also allow studying the disease at its early stages in order to analyse the changes in CSCs long before the cancer can be phenotypically (=clinically) detected.

Besides the therapy per se, an essential element in cancer management is the evaluation of treatment efficacy. Therefore, new clinical methodologies need to be developed to evaluate the efficacy of CSC-based therapies and here again the CSC-based models will be pivotal to achieve this aim.

But perhaps the most crucial question of all is whether hands-off regulation mechanisms can be found in other cancer types, especially tumors of epithelial origin, which represent the bulk of human cancers. Importantly, a small subset of *Sca1-BCR-ABLp210* mice develop additional solid tumors. Considering that Sca1 has been identified as a almost universal stem cell marker in many different tissues, these data suggest that stem cell-driven oncogenesis is not specific to only hematopietic tissues, but rather represents a broader mechanism for deregulation of stem cell differentiation, providing a paradigm that can be applied to solid-organ cancers. Thus, it is to be expected that CSCs from different cancer types will share many similarities, implying that similar CSC-based therapeutic approaches could be used in many different cancers. The challenge is now to find a way to specifically target CSCs and/or the hands-off oncogenic regulation mechanism without causing toxicity to normal cells.

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