

# Genetic background affects susceptibility to tumoral stem cell reprogramming

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The latest studies of the interactions between oncogenes and its target cell have shown that certain oncogenes may act as passengers to reprogram tissue-specific stem/progenitor cell into a malignant cancer stem cell state. In this study, we show that the genetic background influences this tumoral stem cell reprogramming capacity of the oncogenes using as a model the Sca1-BCRABLp210 mice, where the type of tumor they develop, chronic myeloid leukemia (CML), is a function of tumoral stem cell reprogramming. Sca1-BCRABLp210 mice containing FVB genetic components were significantly more resistant to CML. However, pure Sca1-BCRABLp210 FVB mice developed thymomas that were not seen in the Sca1-BCRABLp210 mice into the B6 background. Collectively, our results demonstrate for the first time that tumoral stem cell reprogramming fate is subject to polymorphic genetic control.

## Introduction

Recent contributions have introduced a new perspective on oncogenic transformation, where certain oncogenes may act as passengers to reprogram tissue-specific stem/progenitor cell into a malignant cancer stem cell state.<sup>1,2</sup> The existence of this tumoral stem cell reprogramming as a cancer driver seems to be a common intrinsic mechanism for many types of cancer<sup>3–16</sup> and opens a clear hope for cancer treatment, since epigenetic modifications, unlike genetic changes, can be erased, manipulated, and reinitiated. If we can understand the regulation of the oncogene–target cell interaction, and if, as a result, we learn how to manipulate cellular states experimentally, we could unlock the potential to provide great advances in human cancer medicine.<sup>1</sup> Thus, one of the most essential cellular substratum that would determine cancer behavior and evolution variability among patients would be the reprogramming potential of the oncogenic event. Therefore, we hypothesized that this tumor reprogramming fate at the cancer stem cell (CSC) level should be the target where, at least partly, the action of those genetic determinants takes place, such as modifier genes, which determine the differences among individuals regarding tumor susceptibility, treatment response, and evolution.

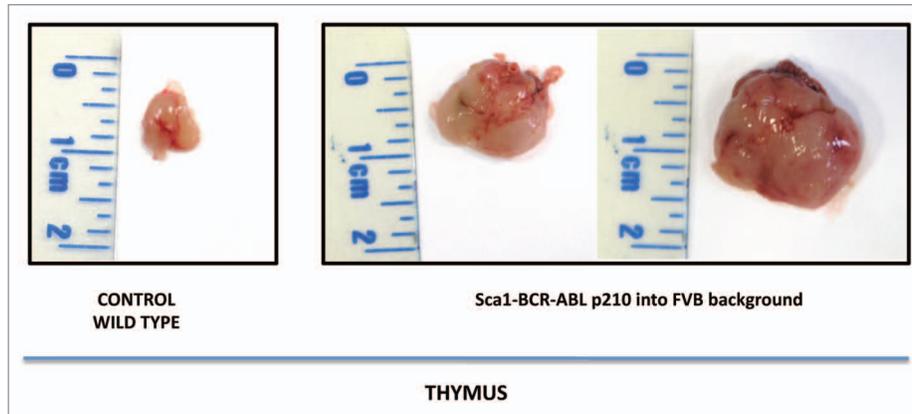
In order to probe whether the genetic background could influence the tumoral stem cell reprogramming fate, we have

used as a model the Sca1-BCR-ABLp210 mice, because the type of tumor that develops is a function of stem cell reprogramming.<sup>4–8,17</sup> In this model the restricted expression of the *BCR-ABL* oncogene, linked to chronic myeloid leukemia (CML) disease, to the hematopoietic stem cell compartment is 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.<sup>4–8,17</sup> *BCR-ABL* oncogene inactivation could not change this tumor reprogramming fate at the CSC level, in agreement with the common occurrence of tumor relapse by which human CML evolves to escape BCR-ABL pharmacological inactivation.<sup>18–21</sup> These observations suggest that the susceptibility to development of CML is intrinsic to the BCR-ABLp210-induced reprogramming of stem cells. As a first step toward identification of genes that control tumor stem cell reprogramming susceptibility, Sca1-BCRABLp210 B6/FVB F1 hybrid mice and pure FVB mice were generated and tested for CML development. The Sca1-BCRABLp210 B6/FVB F1 hybrid mice carry modifier alleles conferring resistance to the development of CML. However, the pure FVB mice carry modifier alleles that result in the rapid development of lethal thymomas. Remarkably, the data provide evidence for the first time that modifier loci would then determine the fate of the oncogene–target cell interaction.

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**Figure 1.** Macroscopic appearance of the thymus in *Sca1-BCRABLp210* mice in FVB background. *Sca1-BCRABLp210* mice in FVB background with signs of disease were analyzed and the thymus was surgically removed. Macroscopically there was sign of thymoma. A thymus of an aged wild-type mouse is shown as a control.

**Table 1.** Genetic background affects stem cell reprogramming in *Sca1-BCRABLp210* mice

Strain	No. of mice	No. with CML (%)	No. with B-cell leukemia (%)	No. with T-cell lymphoma (%)	NO tumors (%)
B6	23	23(100)	0	0	0
B6/FVB (F1)	35	10(28,5)	6(17,2)	0	19(54,3)
FVB	11	0	0	11(100)	0

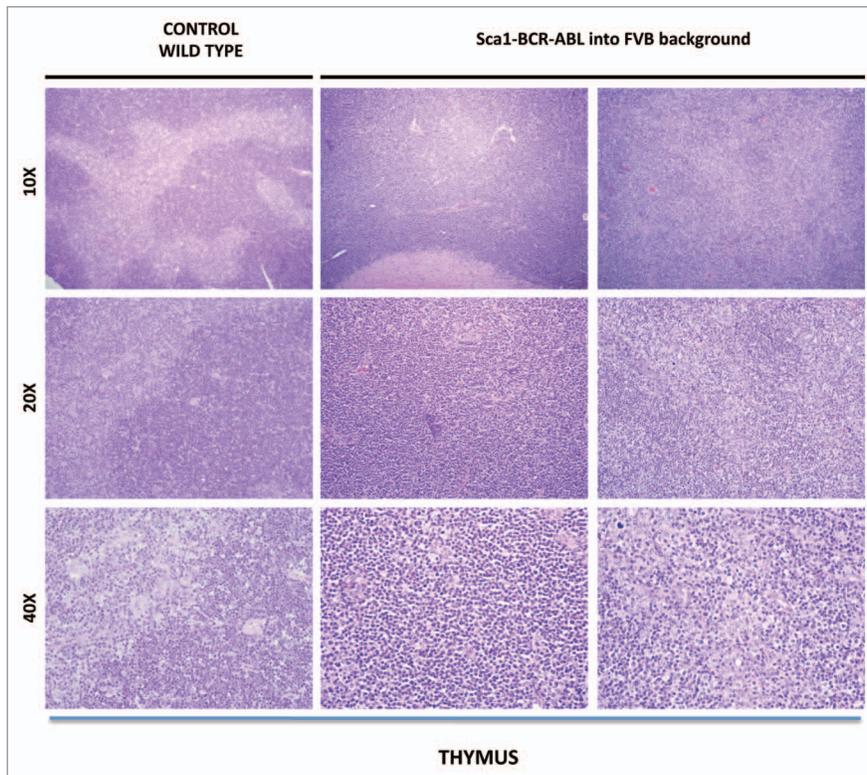
## Results and Discussion

In order to probe if the genetic background could affect the tumoral stem cell reprogramming fate, we have taken advantage of our *Sca1-BCRABLp210* mouse model of human chronic myeloid leukemia (CML). This transgenic mouse was engineered to express the human *BCRABLp210* cDNA under the control of the *Sca1* promoter in order to limit and determine the effect of ectopic expression of *BCRABLp210* in hematopoietic stem/progenitor cells.<sup>4-8,17</sup> This model not only faithfully recapitulates the human disease, but also has been able to anticipate that human CML stem cells' survival is Bcr-Abl kinase-independent, and suggests that curative approaches in CML must focus on kinase-independent mechanisms of resistance.<sup>18-21</sup> Thus, this model represents an ideal system to analyze the contributions of the genetic background to the fate of the interaction of the *BCR-ABLp210* oncogene and the target cell, because the type of tumor that develops is a function of tumoral stem cell reprogramming.<sup>4-8,17</sup> To this end, the *Sca1-BCRABLp210* transgene was moved from the B6 to the FVB genetic background through 6 generations of backcrossing in our laboratory. The resulting strain (FVB, *Sca1-BCRABLp210*) was used for experiments described in this study. B6/FVB F1 mice used in this study were obtained by breeding the FVB, *Sca1-BCRABLp210* mice with regular B6 mice.

In the *Sca1-BCRABLp210* model, the type of tumor that develops is a function of stem cell reprogramming. The modifier loci would then determine the fate of this interaction. Mice were monitored clinically and by serial peripheral blood count for evidence

of CML for 24 months. As described, all B6, *Sca1-BCRABLp210* mice develop CML (Table 1).<sup>4-8,17</sup> Surprisingly, when the *Sca1-BCRABLp210* B6/FVB F1 mice were analyzed, CML was not found in the majority of them, and the survival of these *Sca1-BCRABLp210* B6/FVB F1 mice was significantly increased in comparison with B6, *Sca1-BCRABLp210* mice (Table 1). On the contrary, the majority of old *Sca1-BCRABLp210* B6/FVB F1 mice do not develop CML as evidenced by the normal spleen sizes and normal leukocyte cellularity in the peripheral blood. The absence of CML disease was further confirmed by histologic analysis, which revealed normal spleen where we cannot detect the dramatic expansion of progenitors and differentiated myeloid cells that is characteristic of CML. Quantitative RT-PCR of *BCR-ABLp210* messenger mRNA confirmed that BCR-ABL was expressed in *Sca1-BCRABLp210* B6/FVB F1 hematopoietic stem cells (data not shown). These results indicate that the FVB genetic component in hematopoietic stem cells does interfere with the development of CML induced by BCR-ABL.

In contrast, *Sca1-BCRABLp210* mice inbred into the FVB background are comparatively resistant to the development of CML, as are *Sca1-BCRABLp210* B6/FVB(F1) hybrid mice (Table 1). However, all FVB, *Sca1-BCRABLp210* mice spontaneously developed lymphomas that were not seen in the *Sca1-BCRABLp210* mice, either into the hybrid B6/FVB background or into the B6 background. Lymphomas were observed as early as 16 weeks of age and reached an incidence of 100% by 40 weeks of age (Table 1). FVB, *Sca1-BCRABLp210* mice with lymphomas have typically enlarged thymus comparing to age-matched wild-type controls (Fig. 1). Therefore, genes in the FVB genome can



**Figure 2.** Representative histologic appearance of thymus of diseased *Sca1-BCR-ABLp210* into FVB background and control wild-type mice after hematoxylin-eosin staining. Note the organ infiltration by blast lymphoid cells.

significantly increase the incidence of lymphoma and accelerate the disease when compared with the B6 or FVB/B6 F1 mice. The lymphomas in FVB, *Sca1-BCRABLp210* mice were composed of blastic lymphoid cells that effaced the normal architecture of the thymus. The cellular morphology and the pattern of involvement of lymphoid organs mimic the features of lymphoblastic lymphoma in humans (Fig. 2).

Thymocytes are classified into a number of distinct maturational stages based on the expression of cell surface markers. The earliest thymocyte stage is the double-negative stage (negative for both CD4 and CD8), which can be divided into four sub-stages using CD25 and CD44 markers. The next major stage is the double-positive stage (positive for both CD4 and CD8). The final stage in maturation is the single positive stage (positive for either CD4 or CD8). The analysis of the lymphomas of FVB, *Sca1-BCRABLp210* mice by flow cytometry showed predominantly the presence of CD44<sup>+</sup>CD25<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> double-negative T cells in the thymus (Fig. 3). Thus the genetic background influences the tumoral stem cell reprogramming ability of the *BCRABLp210* oncogene.

Overall, our work demonstrates for the first time that modifier loci would then determine the fate of the tumoral stem cell reprogramming. These studies represent a first step toward the identification of genes that modify stem cell reprogramming susceptibility in *Sca1-BCRABLp210* mice. Because hematopoietic tumors can be quantified and classified relatively easily, the mapping of the modifiers that control stem cell reprogramming

should be possible. Molecular identification of such loci should lead to a better understanding of stem cell reprogramming development. These findings have important implications, as they suggest that it would be possible to prevent cancer to develop once a cancer stem cell has been created.

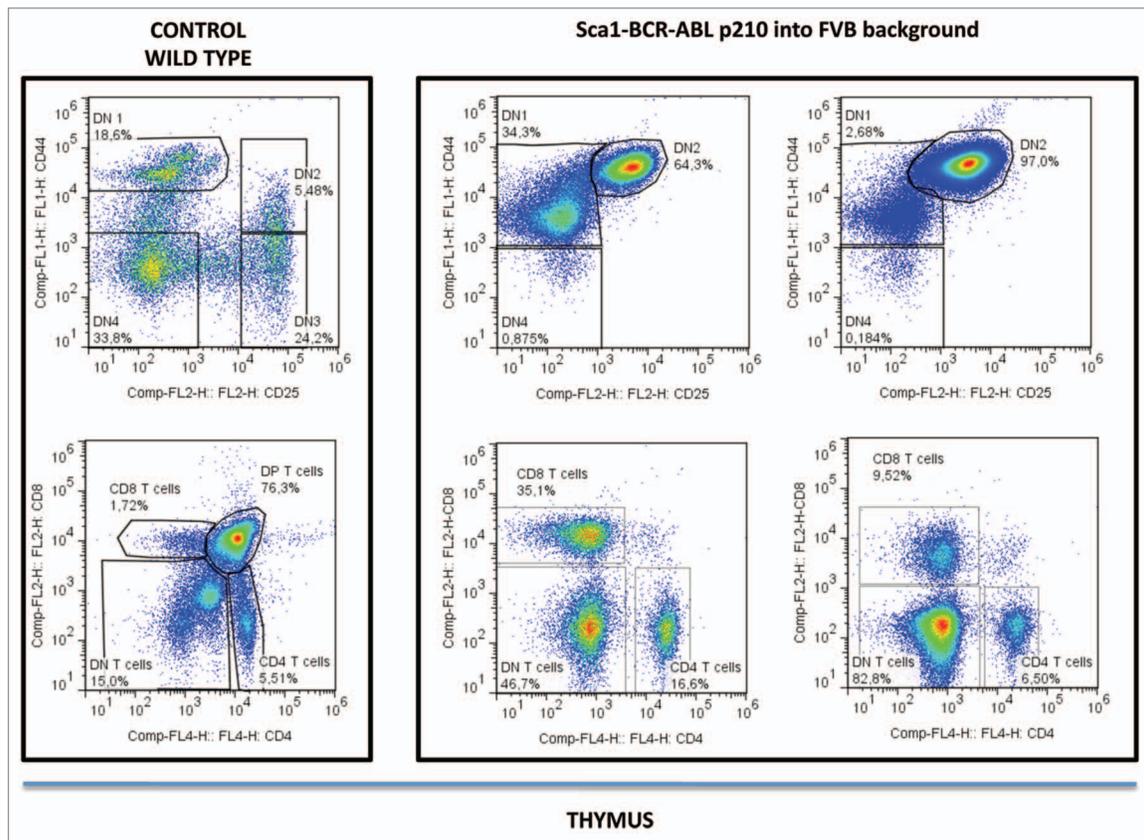
## Materials and Methods

### Ethics statement

All animal work has been conducted according to relevant national and international guidelines and it has been approved by the Bioethics Committee of University of Salamanca and by the Bioethics Subcommittee of Consejo Superior de Investigaciones Científicas (CSIC).

### Mice

The *Sca1-BCRABLp210* transgene was under the control of the *Sca1* promoter as previously described.<sup>4</sup> The transgene was moved from the B6 to the FVB genetic background through 6 generations of backcrossing in our laboratory. The resulting strain (FVB, *Sca1-BCRABLp210*) was used for experiments described in this study. B6/FVB F1 mice used in this study were obtained by breeding the FVB, *Sca1-BCRABLp210* mice with regular B6 mice from the Jackson Laboratory. Mice were monitored for tumors through physical examination. Mice showing sufficient signs of pain and suffering including thymic and cervical lymphoid enlargement were euthanized for a complete necropsy. Final diagnosis of disease was established by clinical, pathologic,



**Figure 3.** Phenotypes of lymphomas in Sca1-BCR-ABLp210 into FVB background. All lymphomas analyzed had a similar phenotype with predominantly CD4<sup>+</sup>CD8<sup>-</sup> double-negative thymocytes. The use of CD25 and CD44 lineage markers allowed to identify these CD4<sup>+</sup>CD8<sup>-</sup> double negative thymocytes as DN2 (CD44<sup>+</sup>CD25<sup>+</sup>) thymocytes.

and FACS analysis. Mice without tumors were monitored for 2 y and then were also euthanized for a complete necropsy.

#### Histological analysis

All mice included in this study were subjected to standard necropsy. All major organs were examined under the dissecting microscope, and samples of each organ were processed into paraffin, sectioned, and examined histologically. All tissue samples were taken by the pathologist from homogenous and viable portions of the resected sample and fixed within 2–5 min. of excision. Hematoxylin and eosin-stained sections of each tissue were reviewed by a single pathologist (OB). For comparative studies, age-matched mice were used.

#### Real-time PCR quantification

cDNA for use in quantitative PCR studies was synthesized using reverse transcriptase (Access RT-PCR System; Promega). Two  $\mu$ l of second round amplified RNA was transcribed. Primers and probes used for quantitative PCR are commercially available (TaqMan Assays-on-Demand Gene Expression Products, Applied Biosystems). In addition the probes were designed so that genomic DNA would not be detected during the PCR. The sequences of the specific primers and probes were as follow: *BCR-ABL*<sup>p210</sup>, sense primer 5'-TTCTGAATGTCATCGTCCACTCA-3', antisense primer 5'-AGATGCTACTGGCCGCTGA-3' and probe 5'-CCACTGGATTTAAGCAGAGTTCAAAAGCCC-3'; *c-Abl*, sense primer 5'-CACTCTCAGCATCACTAAAGTGA

A-3', antisense primer 5'-CGTTTGGGCTTCACACCATT-3', and probe 5'-CCGGTCTTGGGTATAATCACAATG-3'.

#### Analysis and monitoring of disease

Peripheral blood was collected from retro-orbital plexus with a heparinized capillary tube, and total white blood cell and differential counts were performed twice a week. The number of white blood cells was determined with a hemocytometer after lysis of enucleated red blood cells with RCLB lysis buffer (0.15 M NH<sub>4</sub>Cl; 1 mM KHCO<sub>3</sub>; 0.1 mM Na<sub>2</sub>-EDTA, pH 7.4).

#### Flow cytometry

Nucleated cells were obtained from total bone marrow (flushing from the long bones), peripheral blood, thymus, liver, and spleen. In order to prepare cells for flow cytometry, contaminating red blood cells were lysed with RCLB lysis buffer and the remaining cells were then washed in PBS with 2% FCS. After staining, all cells were washed once in PBS with 2% FCS containing 2 mg/mL propidium iodide (PI) to allow dead cells to be excluded from both analyses and sorting procedures. Monoclonal antibodies were obtained from PharMingen and included: lineage markers (CD45R/B220, for B lineage staining; CD4, CD8 and CD44 and CD25 for T cell lineage; CD11b and Gr1 for myeloid lineage and TER119 for erythroid lineage) and Sca1 (E13–161.7) for stem cells. Single-cell suspensions from the different tissue samples obtained by routine techniques were incubated first with purified anti-mouse

CD32/CD16 (PharMingen) prior to the addition of other antibodies, to block binding via Fc receptors and then with an appropriate dilution of the different antibodies at room temperature or 4 °C, respectively. The samples and the data were analyzed in a FACSCalibur using CellQuest software (Becton Dickinson). Specific fluorescence of FITC and PE excited at 488 nm (0.4 W) and 633 nm (30 mW), respectively, and known forward and orthogonal light-scattering properties of mouse cells were used to establish gates. For each analysis a total of at least 5000 viable (PI<sup>-</sup>) cells were assessed.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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