

# Tumor Growth Need Not Be Driven by Rare Cancer Stem Cells

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Cancer biologists are intrigued by the hypothesis that tumor growth may be sustained by a rare subpopulation of the cells, termed cancer stem cells. Supporting this concept are the heterogeneous cellular composition of certain tumors and the finding that only a minute proportion of the cells ( $\sim 1/10^6$ ) in some human acute myeloid leukemia (AML) samples can seed tumor growth when transplanted into sublethally irradiated nonobese diabetic (NOD) severe combined immunodeficient (*scid*) mice (1). The interpretation of such xenotransplantation studies, however, is complicated by the critical role in tumor growth of interactions with the microenvironment, which are mediated by both soluble and membrane-bound factors (2). Notably, many such mouse factors cannot engage the cognate human receptor and vice versa (3). Thus, the low frequency of human AML cells producing tumors in NOD/*scid* mice might reflect in part the rarity of human tumor cells that can readily adapt to growth in a foreign (mouse) milieu.

In our view, the frequency of cells that can sustain tumor growth, and thus the generality of the cancer stem cell hypothesis, can best be tested by transfer of titrated numbers of mouse tumor cells into nonirradiated histocompatible recipient mice. We isolated primary pre-B/B lymphoma cells from three independent  $E\mu$ -*myc* transgenic

mice and injected 10 to  $10^5$  cells into non-irradiated congenic animals. Regardless of the cell number injected, all recipients became moribund with disseminated lymphoma within 35 days (Table 1). Although the number of injected cells did not noticeably affect tumor burden, organ infiltration, or disease severity, recipients of 10 or 100 lymphoma cells usually developed tumors more slowly than those receiving  $10^5$  cells. Importantly, even transfer of a single cell elicited fatal lymphoma in three of eight recipients within 33 to 76 days (case 2).

A small fraction ( $\sim 2$  to 5%) of the cells in primary  $E\mu$ -*myc* lymphomas displayed the characteristic stem cell markers Sca-1 and/or AA4.1. However, when sorted Sca-1<sup>+</sup> AA4.1<sup>hi</sup> or Sca-1<sup>+</sup> AA4.1<sup>lo</sup> lymphoma cells were transplanted, as few as 10 cells of each population elicited fatal lymphoma within 17 to 40 days (Table 1). Similarly, with  $E\mu$ -*N-RAS* thymic lymphomas and four independent cases of AML caused by PU.1 deficiency, recipients transplanted with as few as 10 cells developed tumors, although onset was delayed in mice receiving only 10 or 100 AML cells (Table 1). For all three malignancies, the cell surface marker phenotype (fig. S1), the gene expression profile (fig. S2), and the invasiveness of the transplanted tumors mirrored that of the primary tumor.

**Table 1.** A large proportion of tumor cells can sustain the growth of murine lymphoid and myeloid malignancies. Cells from primary  $E\mu$ -*myc* pre-B/B lymphomas,  $E\mu$ -*N-RAS* thymic lymphomas, or  $PU.1^{-/-}$  AML, all from mice on a C57BL/6 (Ly5.2<sup>+</sup>) background ( $>15$  backcrosses), were transplanted into nonirradiated congenic C57BL/6 (Ly5.1<sup>+</sup>) recipient mice. To circumvent problems associated with injection of low cell numbers, we mixed the tumor cells with  $10^6$  congenic (C57BL/6-Ly5.1<sup>+</sup>) spleen cells as carriers. Shown are the fraction of recipients that developed tumors and the average time from transplantation to tumor development. No mice (0/24) injected with carrier spleen cells alone developed any tumor over a 100-day period. ND, not determined.

	Recipients that developed tumors (days to kill)			
	$10^5$ cells	$10^3$ cells	$10^2$ cells	10 cells
<i>Eμ-myc B lymphoma</i>				
Case 1	3/3 (25)	3/3 (25)	3/3 (32)	2/2 (35)
Case 2	3/3 (21)	3/3 (23)	3/3 (24)	3/3 (24)
Case 3 Sca-1 <sup>+</sup> AA4.1 <sup>hi</sup>	3/3 (21)	3/3 (21)	ND	3/3 (17)
Sca-1 <sup>+</sup> AA4.1 <sup>lo</sup>	2/2 (17)	2/2 (28)	2/2 (28)	2/2 (40)
<i>Eμ-N-RAS T lymphoma</i>				
Case 1	3/3 (28)	3/3 (42)	3/3 (28)	3/3 (28)
<i>PU.1<sup>-/-</sup> AML</i>				
Case 1	1/1 (54)	2/2 (168)	1/2 (192)	0/2
Case 2	2/2 (84)	2/2 (85)	2/2 (224)	1/2 (114)
Case 3	1/1 (85)	2/2 (62)	2/2 (69)	2/2 (90)
Case 4	1/1 (30)	1/1 (37)	2/2 (79)	2/2 (88)

These observations challenge the concept that growth of AML, and possibly other malignancies, are always sustained by a rare cancer stem cell (1). Although cancer stem cells may well drive the growth of many cancers, particularly those displaying extensive differentiation, our studies of mouse lymphomas and leukemias indicate that at least certain malignancies (particularly those with substantial homogeneity) can be maintained by a relatively large proportion ( $>10\%$ ) of tumor cells, perhaps even the majority. Although mouse and human tumors differ in notable respects, the marked disparity with results from human AML cells (1) suggests that xenotransplantation may underestimate the percentage of tumor-sustaining cells. With common human solid tumors (for example, brain, colon, and breast), transplantation places the tumor growth-sustaining cells within subpopulations (for example, CD133<sup>+</sup>) that compose up to 20% of the cells (4–6), and most of the remaining cells might be at differentiation stages unsupportable by the mouse microenvironment. The reported rarity of cancer stem cells in AML (1) and colon cancer (4) might reflect the need to cotransfer an essential human accessory cell (we note that endothelial cell progenitors are also CD133<sup>+</sup>).

Determining whether the growth of various tumors is sustained by most of the tumor cells or by a rare subpopulation has important ramifications for the design of novel therapies. Therefore, the cancer stem cell hypothesis merits more rigorous tests. For human tumors, ultimately this will require transfer of tumor cells into mice installed with all the requisite human support cells. Lastly, because the term “cancer stem cell” also currently designates the normal cell that founded the tumor, we suggest that the cells sustaining growth of an established tumor be referred to as “tumor-propagating cells.”

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## Supporting Online Material

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Figs. S1 and S2  
References and Notes  
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