Supporting Online Material for

Comment on “Tumor Growth Need Not Be Driven by Rare Cancer Stem Cells”

James A. Kennedy, Frédéric Barabé, Armando G. Poepl, Jean C. Y. Wang, John E. Dick*

*To whom correspondence should be addressed. E-mail: jdick@uhnres.utoronto.ca

Published 14 December 2007, Science 318, 1722c (2007)
DOI: 10.1126/science.1149590

This PDF file includes:
- Materials and Methods
- Table S1
- References
Supporting Online Material

Materials and Methods

Transduction, transplantation and analysis of leukemic mice

Cord blood samples were obtained according to procedures approved by the institutional review boards of the University Health Network and Trillium Hospital. Samples were pooled and Lin-CB cells were purified by negative selection (StemSep Human Progenitor Enrichment Cocktail, Stem Cell Technologies) and infected as previously described, using viral particles generated from a MSCV-MLL-ENL-murine pgk-EGFP vector (leukemia 1) or a MSCV-MLL-ENL-human pgk-EGFP vector (leukemia 2) (S1). Following transduction, 2.5 x 10^5 cells were injected intrafemorally into B-NOD/SCID mice that had been irradiated with 210 cGy 24 hours previously. Primary mice were sacrificed when overtly ill and bone marrow (BM) was harvested from the 2 femurs, the pelvis and the right tibia. Secondary transplantation was performed as above, using varying doses of the bulk leukemic BM, or the indicated numbers of sorted cells. Secondary mice were sacrificed when moribund, or at 20 weeks post-transplantation. Animals were not carried longer to avoid the confounding influence of thymoma development. At the time of sacrifice the BM, spleen and occasionally the thymus and peripheral blood were analyzed by flow cytometry to assess human engraftment.

Flow cytometry

Flow cytometric analysis was performed on a Becton Dickinson FACSCalibur™ and surface markers were detected with fluorescent human-specific antibodies from Becton Dickinson (anti-CD19 PE, anti-CD34 APC, anti-IgM PC5, anti-IgD PE) and from Beckman Coulter (anti-CD20 PC5, anti-CD33 PC5, anti-CD45 PE, anti-CD45 PC5, anti-CD45 APC).

In order to determine the phenotype of L-IC in our model of MLL-induced B-ALL, BM cells from a primary MLL-ENL mouse were stained with anti-CD19 PE and anti-CD34 APC. Using a Becton Dickinson FACSaria™, GFP+ cells were sorted into 4 fractions based on CD19 and CD34 expression then were injected into mice at the indicated doses. Purity was 99.8% for the GFP+CD19+CD34- fraction, 98.8% for the GFP+CD19+CD34+ fraction (with 1% contamination by CD19+CD34- cells), and was not assessed for the remaining fractions due to low cell numbers.

Statistical Analysis

The data from limiting dilution experiments was analyzed by applying Poisson statistics to the single hit model as previously described (S2). L-IC frequency was calculated using the maximum likelihood estimator.
<table>
<thead>
<tr>
<th>CELL DOSE</th>
<th>BULK LEUKEMIA</th>
<th>GFP+ 19+ 34+</th>
<th>GFP+ 19+ 34-</th>
<th>GFP+ 19- 34+</th>
<th>GFP+ 19- 34-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of leukemic mice</td>
<td>Median disease latency</td>
<td># of leukemic mice</td>
<td>Median disease latency</td>
<td># of leukemic mice</td>
</tr>
<tr>
<td>125 000</td>
<td>2/2</td>
<td>57</td>
<td>2/2</td>
<td>60.5</td>
<td>-</td>
</tr>
<tr>
<td>25 000</td>
<td>2/2</td>
<td>64</td>
<td>2/2</td>
<td>67</td>
<td>-</td>
</tr>
<tr>
<td>5 000</td>
<td>5/5</td>
<td>85</td>
<td>4/4</td>
<td>82.5</td>
<td>-</td>
</tr>
<tr>
<td>1 000</td>
<td>4/4</td>
<td>92</td>
<td>5/5</td>
<td>98</td>
<td>0/3</td>
</tr>
<tr>
<td>200</td>
<td>4/4</td>
<td>125</td>
<td>5/5</td>
<td>101</td>
<td>0/2</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Table S1: Phenotypic characterization of L-ICs in *MLL-ENL* B-ALL.

Leukemia was defined as ≥ 20% CD19⁺CD20⁻ blasts in the BM.
Acknowledgments

We gratefully acknowledge F. Notta for technical assistance and thank B. Neel and members of the Dick lab for critical comments on the manuscript. This work was supported by a Canadian Institute of Health Research MD/PhD studentship (JAK), and grants from the Canadian Institute of Health Research, Ontario Cancer Research Network, Ontario Institute for Cancer Research with funds from the Province of Ontario, Genome Canada through the Ontario Genomics Institute, a Canada Research Chair, the Leukemia and Lymphoma Society, and the National Cancer Institute of Canada with funds from the Canadian Cancer Society and the Terry Fox Foundation (JED).

References
