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## Response to Comment on "Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells"

Both our study (1) and that of Krause *et al.* (2) clearly demonstrated that a single bone marrow (BM)-derived cell can fully reconstitute the hematopoietic compartment of lethally irradiated adult mice. However, these studies differed significantly in their assessment of the ability of the transplanted cell to additionally give rise to nonhematopoietic cell fates.

Whereas Krause *et al.* found substantial contributions of donor-derived cells to both hematopoietic and nonhematopoietic tissues, we observed robust engraftment of the hematopoietic compartment (our mice showed ~16, 30, 40, or 60% donor-derived green fluorescent protein (GFP)-positive peripheral blood leukocytes at the time of sacrifice) but only rare contributions to nonhematopoietic tissues (seven hepatocytes and a single Purkinje cell). The extremely low level of incorporation of c-kit<sup>+</sup>Thy1.1<sup>lo</sup>Lin<sup>-lo</sup>Sca-1<sup>+</sup> (KTLS) hematopoietic stem cell (HSC)-derived cells—or genetic markers of these cells—into nonhematopoietic tissues strongly suggests that these cells show little developmental plasticity. Importantly, our study made no claims regarding the mechanism or mechanisms by which the rare GFP<sup>+</sup> HSC-derived hepatocytes and Purkinje cell arose. It remains unknown whether these cells represent a true "transdifferentiation" event, particularly in light of recent reports demonstrating the capacity for spontaneous fusion of mature and immature cell types (3, 4). Further studies will be required to properly evaluate the biological processes that underlie the generation of these cells.

As stated explicitly in (1), our study does not exclude the possibility that other non-HSC, stem/progenitor populations within BM can contribute to nonhematopoietic tissues. The apparent discrepancy between our results and those in (2) regarding the nonhematopoietic potential of HSCs most likely relates to differences in the cell populations analyzed. We agree with Theise *et al.* (5) that fundamental differences in cell isolation methods likely led to the recovery of nonidentical BM populations, which may differ substantially in their biological properties. Indeed, phenotypic analysis of the quiescent, marrow-homing Lin<sup>-</sup>FR25 marrow cells isolated by

Krause *et al.* showed heterogeneous expression of the cell surface marker Sca-1 (2), a direct demonstration that this population is distinct from the uniformly Sca-1<sup>+</sup> KTLS HSCs that we analyzed. Previous transplantation studies have indicated that all the hematopoietic reconstituting activity in normal mouse BM resides in the KTLS population (6–8), and that all reconstituting activity in the elutriated, FR25 population of C57Bl/Ka-Thy1.1 mouse BM resides in the Thy1.1<sup>lo</sup>Lin<sup>-lo</sup>Sca-1<sup>+</sup> subset (9, 10). However, the BM homing protocol employed in (2) may have allowed the selection of a rare population that maintains regenerative potential for other tissue types, and was not detected following direct transplantation of single-marker selected BM populations due to its very low frequency in normal, unselected bone marrow. Thus, our data are not directly comparable to those of Krause *et al.* and do not implicitly refute their observations.

Theise *et al.* (5) suggest that insufficient sensitivity of our detection method may also have contributed to our failure to identify substantial nonhematopoietic engraftment following HSC transplantation. In particular, they suggest that GFP expression from the transgenic  $\beta$ -actin promoter may be silenced following transplantation, and cite data from a different transgenic line—Rosa26—which shows less than ubiquitous expression of  $\beta$ -galactosidase in donor-derived cells following transplantation. However, the capricious nature of transgene expression in Rosa26 mice does not necessarily indicate that this same problem plagues all transgenic animals. Differences in the transgenic construct or its integration site may differentially affect transgene expression. As shown in figure 3 in (1), transgenic expression of GFP from the  $\beta$ -actin promoter was easily detectable in all cells and tissues of our donor mice, and was particularly high in the epithelial tissues of the gut and lung. It was also detectable in rare nonhematopoietic cells (hepatocyte or Purkinje cell) derived from single transplanted GFP<sup>+</sup> KTLS HSCs. Furthermore, using the parabiotic mouse model, we similarly failed to detect high-level contribution by any circulating stem/progenitor cells to nonhematopoietic tissues, despite substan-

tial chimerism of BM and BM HSCs. Amplification of the GFP signal by immunofluorescent staining with anti-GFP antibodies did not alter these results (1). Finally, our analysis of donor-derived peripheral blood leukocytes in recipients of CD45 congenic, GFP transgenic HSC transplants did not show loss of GFP expression within the highly engrafted hematopoietic compartment of recipient mice (11). Thus, although we cannot formally exclude a specific loss of GFP expression by putative HSC-derived epithelial cells following HSC transplantation, we feel that transgene silencing is unlikely to explain the quantitative differences in the results of our two studies. Whereas the detection method employed by Krause *et al.* clearly is not susceptible to transplantation-induced "silencing," their analysis of donor Y chromosomes required correction of the engraftment rate due to incomplete nuclear sampling. Also, it is possible that some Y signals from infiltrating hematopoietic cells could be attributed to donor-derived epithelial cells, thereby overestimating donor cell engraftment of nonhematopoietic tissues. More likely, however, as discussed above, the disparity in our results relates to fundamental differences in the cell populations analyzed. In future experiments, it shall be of substantial interest to determine the relationship between KTLS HSCs and the quiescent, BM-homing Lin<sup>-</sup>FR25 cells isolated by Krause *et al.* (2).

Amy J. Wagers  
Richard I. Sherwood  
Julie L. Christensen  
Irving L. Weissman

Department of Pathology and  
Department of Developmental Biology  
Stanford University School of Medicine  
279 Campus Drive  
Stanford, CA 94305, USA  
E-mail: awagers@stanford.edu

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