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No Monkeying Around: Clonal Tracking of Stem Cells and Progenitors in the Macaque

Brad Dykstra[1,*] and Leonid V. Bystrykh[2,*]
[1]Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School, Boston MA 02115, USA
[2]Laboratory of Ageing Biology and Stem Cells, European Research Institute for Biology of Ageing, University Medical Center Groningen, University of Groningen, 9700 AD, Groningen, The Netherlands
*Correspondence: bdykstra@partners.org (B.D.), l.bystrykh@umcg.nl (L.V.B.)
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Clonal tracking of hematopoietic stem and progenitor cells (HSPCs) has proven valuable for studying their behavior in murine recipients. Now in *Cell Stem Cell*, Kim et al. (2014) and Wu et al. (2014) extend these analyses to nonhuman primates, providing insights into dynamics of HSPC expansion and lineage commitment following autologous transplantation.

Hematopoietic stem cells (HSCs) are remarkable in their ability to ultimately produce billions of blood cells a day for a lifetime, without exhaustion. Several decades ago, transplantation experiments using chromosomally marked cells identified unique patterns of stem cell regeneration and also demonstrated conclusively that lymphoid and myeloid cells could be derived from a single cell (Abramson et al., 1977). Since then, technological advances have allowed more precise interrogation of individual hematopoietic stem and progenitor cells (HSPCs). In the mouse, concepts of stem cell heterogeneity are now well established (Copley et al., 2012), and the hematopoietic hierarchy has been found to be more complicated than originally thought (Kawamoto and Katsura, 2009). Recent studies using human cells xenotransplanted into immunodeficient mice have revealed similarities in human hematopoiesis (Doulatov et al., 2010; Görgens et al., 2013). However, differences in niche components, cytokines, body temperature, life span, and body size place artificial constraints and demands onto primate hematopoietic cells engrafted into murine hosts. Thus, uncertainty remains as to the applicability of the xenotransplant system to inform regarding primate hematopoietic biology in the setting of transplantation.

In this issue of *Cell Stem Cell*, two papers report tracking thousands of individual marked HSPCs in nonhuman primates. The first paper (Kim et al., 2014) tracked unique vector integration sites to measure short- and long-term clonal output of HSPCs in several autologously transplanted macaques for up to 12 years. The second paper (Wu et al., 2014) used a lentiviral barcoding approach to track the clonal origins of multiple differentiated cell types produced in the first 1–9 months posttransplantation in similarly transplanted macaques. These two complementary studies provide the first data set of this type in primates, providing valuable clonal contribution and lineage specification data for autologously transplanted primate HSPCs.

The long-term clonal analysis reported by the Chen group (Kim et al., 2014) revealed an initial stage of clonal fluctuation for the first 6–12 months posttransplantation, after which clonal contributions largely stabilized, with waves of clones expanding and contracting over a longer period of time. The observation that clonal stability was not observed until at least 1 year posttransplant indicates that, in this setting, the fluctuations seen early after transplantation likely reflect behaviors of progenitors rather than stem cells. Most long-term clones, defined as those persisting for 3–10 years, were undetectable at 2–4 months and were only minor contributors of blood cells until 7–13 months posttransplantation, after which they became the primary source of circulating blood cells. Within the long-term HSC clones, the authors observed myeloid-biased, lymphoid-biased, and balanced lineage outputs, with the balanced HSCs becoming the predominant source of hematopoietic reconstitution over the longterm. Importantly, the authors also compared the clones observed in CD34+ HSPCs isolated from the bone marrow several years posttransplant with those observed in the blood at a similar time point, and found high overlap with the balanced and myeloid-biased clones, but a lower overlap with the lymphoid-biased clones. This suggests that at least some of the lymphoid-biased clones present in peripheral blood were not a result of active hematopoiesis within the bone marrow, but instead were a remnant of long-lived lymphoid cells from an exhausted clone.

In contrast, the report by the Dunbar group (Wu et al., 2014) interrogates a relatively short period of time posttransplantation (4.5, 6.5, and 9.5 months in three transplanted animals). This encompasses the time period classified by Kim et al. as predominantly/exclusively short-term reconstitution (between 7 to 9 months) and suggests that Wu et al. are analyzing primarily hematopoietic progenitor cells, since the downstream progeny of true long-term HSCs would only have just begun to appear at this time. Clonal measurements of lineage output in the period between 1 and 6 months posttransplant indicated an initial wave of unilineage progenitors, followed by successive waves of granulocyte/monocyte (termed myeloid, M), myeloid/B cell (M/B), and finally myeloid/B cell/T cell (M/B/T) progenitors. Of interest, the barcodes seen in the myeloid and B cells at a given time point seemed to correlate better than those in T cells and B cells alone, indicating output from M/B or M/B/T progenitors, but not common lymphoid (B/T) progenitors. This is consistent with recent findings in vitro and in immunodeficient mice (Doulatov et al., 2010), and it is further evidence that a

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strict early bifurcation of lymphoid and myeloid lineage potential is likely not the case in primates.

Perhaps the most striking observation was the distinct clonal distribution of natural killer (NK) cells. There are two main NK cell subsets: the cytotoxic $CD56^{dim}CD16^{+}$ NK cells, found primarily in the blood, and the cytokine-producing $CD56^{bright}CD16^{-}$ NK cells, found primarily in the lymphoid organs. The clonal distribution of the $CD56^{bright}$ NK cells generally correlated with M/B/T lineages. However, the main population of NK cells in the blood ($CD56^{dim}CD16^{+}$) did not correlate with any other cell type, suggesting that this NK subset had an entirely different ontogeny. This is in contrast to the generally accepted notion that $CD56^{bright}CD16^{-}$ NK cells are precursors of $CD56^{dim}CD16^{+}$ cells (Romagnani et al., 2007). Furthermore, NK clonal distribution remained distinct from T and B cell restricted clones, but correlated instead with M/B and M/B/T clones. This observation calls into question yet another aspect of the traditional hematopoietic hierarchy model, which almost invariably considers NK cells as progeny of common lymphoid progenitors (CLPs). The data here strongly suggests that these two NK subsets have separate origins and do not arise exclusively from CLPs, at least not in a transplantation setting.

Integration-site-based methods of clonal analysis have been used for more than 2 decades, and as such many of the technical challenges of this approach have been discussed in detail. In contrast, the barcoding method is relatively new on the scene, and the technical caveats are still being worked out. Initial design and validation to determine size and skewing of the barcode library is critical, as is dealing with sequencing error and setting appropriate thresholds to minimize false-positives and false-negatives. Both methods used here to some degree encounter similar challenges of calibration and noise discrimination that cannot be ignored (Bystrykh et al., 2012). Since both Kim et al. and Wu et al. cotransplanted a large number of HSPCs per recipient, inevitably many smaller clones are at the edge of detection limits in the blood. Due to the inherent challenges of discriminating these small signals from sequencing noise, the reliability of measurement decreases dramatically proportional to clone size. Therefore, the frequencies of repopulating clones reported here should be taken as general estimates at best. Furthermore, it is important to consider the major difference in the timing and duration of clonal tracking (i.e., months versus years) when comparing the data and conclusions of the two papers. Although similar terminology (such as short-term and long-term, lineage-biased, and stability) is used in both papers, they are not necessarily referring to the same thing. In any case, both of these studies provide exciting new insights on the nature of hematopoietic reconstitution in a clinically relevant transplantation system.

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