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Bridge Over Troubled Stem Cells

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Over the five decades since their first definitive identification,[1,2] hematopoietic stem cells (HSCs) have emerged as the most clinically exploited somatic stem cell population, with more than 55,000 bone marrow transplants (autologous and allogeneic combined) performed worldwide in 2009, including about 20,000 in the United States alone.[3] Our inability to directly identify human HSCs among progenitors of more limited potential has hampered high-resolution molecular analysis of human long-term HSCs (LT-HSCs), which is the key to unlocking their clinical and therapeutic potential and bridging the gap between suitable stem cell supply and demand. A recent xenograft study reported by Notta *et al.* in *Science*[4] has brought one step closer the possibility of modulating human LT-HSCs *ex vivo* for clinical therapies.

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Bone marrow transplantation has become the standard of care for many malignant and nonmalignant hematopoietic diseases, including Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, acute leukemia, chronic leukemia, aplastic anemia, and myelodysplastic syndromes. Despite this clinical success, the demand for compatible transplant marrow far outweighs the supply of suitable donor material. Efforts to bridge this disparity have led to experimental studies to better identify and expand the most important human HSC subsets. The mouse has served as the most widely used experimental model system for studying HSC biology. A vast array of markers have been described that can be used in flow-cytometric sorting to obtain populations of mouse bone marrow cells that are highly enriched for HSCs.[5–11] This has led to many elegant molecular studies of highly purified mouse HSCs, yielding tremendous insight into the mechanisms that empower their unique characteristics. However, the same cannot be said for human HSC research. The markers used for segregation of true murine LT-HSCs from short-lived or lineage-restricted progenitors are not necessarily conserved between mice and humans.

Notta *et al.*[4] used a mouse model to identify a population of human cells with the phenotype of $CD34^+CD38^-CD45RA^-Thy1^+Rho^{lo}CD49f^+$, which was highly enriched for long-term *in vivo* HSC activity at the single-cell level. One of the major milestones in this paper was the delineation of true human stem cell activity from that of multipotent progenitors that are able to give rise to multilineage differentiation *in vivo*, albeit only transiently (most activity gone by 10 weeks post-transplant). Discrimination of human cell populations with different *in vivo* functional potential using their new markers and this sensitive transplantation assay will allow for powerful molecular analysis of highly enriched cell populations (**Figure 1**).

Ultimately, HSCs are defined by function, not by phenotype, and the gold standard for *in vivo* HSC activity is bone marrow transplantation. The operational definition of an HSC in general terms is the ability of a cell to repopulate a recipient mouse with long-term (>4 months) multilineage reconstitution, with a single clone contributing to myeloid, B-, and T-cell lineages. In the past, clonality was examined by specific chromosomal translocations, then by unique retroviral integration sites. More recently, the "platinum standard" has been to transplant mice with a single cell. With the highest-purity murine stem cell populations, around one in three to one in five of transplanted mice will show multilineage blood contribution with a single HSC.[6,9,12–15]

Although the studies described above have allowed refinement of the phenotypic definition of HSCs and enabled markers to be identified that allow separation of HSC subtypes,[11,14,15] similar progress in human HSC research has lagged behind. Human HSCs have been defined by either *in vitro* activity or transplantation into mice. Over the past 20 years, several mouse strains have been developed and tested for their ability to accept human hematopoietic grafts. Although many immunocompromised mouse strains will support some human hematopoietic development, the various models have supported some lineages better than others, making it difficult to discern true HSC quality differences. Over time, the use of the various strains has become more refined with the use of severely immunocompromised recipient mice such as

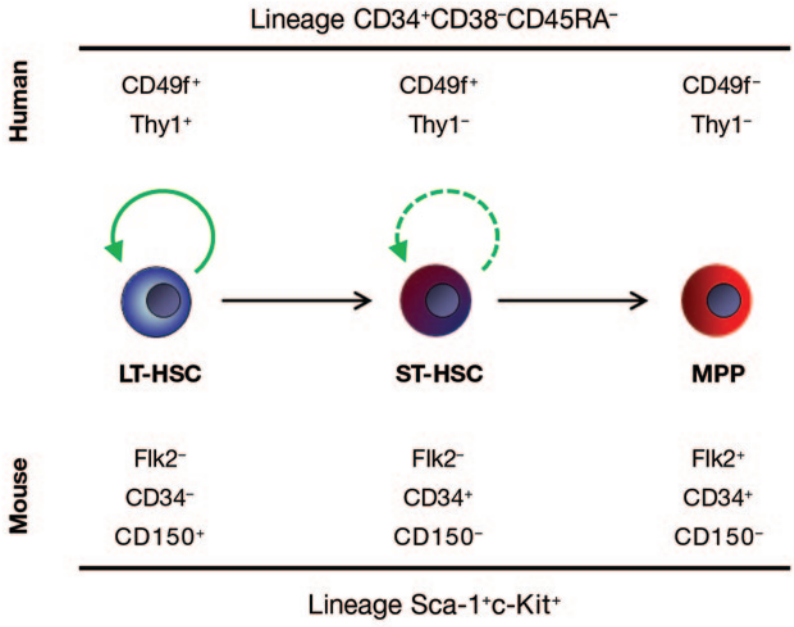

Figure 1 Cell surface markers used for segregation of human and mouse LT-HSCs from ST-HSCs and multipotent progenitors. Green arrows represent self-renewal potential, with the long-term hematopoietic stem cells (LT-HSCs) possessing durable self-renewal potential and the short-term HSCs (ST-HSCs) showing more limited capacity. Multipotent progenitors (MPPs) have no long-term self-renewal *in vivo*.

NOD-*scid*-IL2Rγ$^{-/-}$ (NSG). NSG mice lack mature T cells, B cells, and functional natural killer cells and are deficient in cytokine signaling, providing the most permissive environment for engraftment of human donor cells. Although these refinements have markedly improved sensitivity of engraftment into mice, they restrict the techniques almost exclusively to the hands of the most expert labs because the immunocompromised mice are expensive to maintain and the experiments technically demanding. Nevertheless, these studies are essential, for even though the mouse HSC has served as the model of choice for experimental hematology, many of the findings cannot be extrapolated to human HSCs, including, notably, the cell surface markers used for HSC purification (**Figure 1**).

The study by Notta *et al.*[4] represents a landmark because it combined the latest and best xenotransplantation strategies with the newest human HSC markers to achieve remarkably robust long-term, multilineage engraftment from transplantation of highly purified HSCs. The authors performed intrafemoral injections of purified cell populations into NSG mice,[4] thus avoiding the potential complications due to HSC homing that arise from the typical injection of donor cells into the circulatory system either retro-orbitally or via the tail vein. By doing this, they could demonstrate robust chimerism of human cells in the hematopoietic system (blood, bone marrow, spleen, thymus) at 20 weeks post-transplant with contribution to erythroid, B-lymphoid, myeloid, and T-lymphoid lineages. They ultimately applied the platinum standard and transplanted single human HSCs into the mice, observing multilineage engraftment from 14 to 28% of individual human HSCs. These cells were also capable of repopulating secondary hosts, indicating extensive self-renewal ability.

The authors did note some variability in HSC frequency between experiments that they attribute to the genetic heterogeneity of different cord blood donors.[4] Although this study did not demonstrate the level of HSC frequency seen in clonal transplantation of highly purified mouse HSC fractions, it represents a remarkable technical feat achieved through years of refining techniques. The technical challenges associated with single-cell transplantation (maintenance of cell viability during sorting, ensuring that the test cell is actually contained within the injection bolus, correct placement of injection site) probably result in underestimation of the HSC frequency in such studies. In addition, the technical challenges associated with this system may prevent most labs from using it as a routine assay. Thus, although this study sets a new standard, most other human HSC work should not be required to match this, and, indeed, a test of function on a clonal level is not needed in most experimental settings.

A devil's advocate could argue that xenograft transplantation of human cells into mice may not reflect the true properties of human HSCs; the assay may best identify cells that can survive and proliferate in response to murine cytokines in a foreign environment rather than native human HSCs. Although the general bias of the field is that an *in vivo* transplantation experiment, however foreign, is better than an *in vitro* assay, we cannot really know how well this simulates transplantation of marrow and cord blood into human patients afflicted with hematological diseases. The dearth of reliable assays to test the activity of human cells remains a major impediment to much of cell therapy (and stem cell) research. Although more work is needed to determine whether the cells identified by Notta *et al.* represent the real HSCs responsible for repopulating transplant patients, the study nonetheless represents a remarkable technical achievement and enables us to further investigate an important candidate stem cell. The challenge will now be to direct these cells into therapeutically useful applications such as *ex vivo* expansion and gene therapy.

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