SPECIAL REPORT

Reconsidering marrow stem cell cycle status: insights into an actively cycling hematopoietic stem cell population

LR Goldberg

Leukemia Supplements (2014) 3, S12–S13; doi:10.1038/leusup.2014.7**Keywords:** highly purified stem cells; quiescence; cycling stem cells; stem cells heterogeneity; stem cell markers

Several decades of research in the hematopoietic stem cell (HSC) field have led to increasingly detailed characterizations of HSC phenotype, HSC niches, and the molecular pathways governing stem cell self-renewal and differentiation fate. One of the unifying principles of virtually all studies to date has been the focus on highly purified stem cell populations, a process that has been achieved primarily through the isolation of lineage-negative cells with unique supravital dye staining characteristics and distinguishable cell surface phenotypes, including positivity for c-Kit, Sca-1 and CD150, and negativity for Flk-2 and CD34.^{1–5} Elegant studies have shown that, by using these separative techniques, one can purify very small subpopulations of marrow cells with tremendous differentiation and renewal potential. Cell cycle analyses have identified these purified stem cells to be predominantly in a quiescent state.^{6–8} It is this highly purified, quiescent subset of marrow cells that has dominated the stem cell field, forming the core of most research to date. In contrast, we have been exploring the stem cell potential of unseparated whole bone marrow and have found that long-term multi-lineage engraftable cells in unseparated marrow are actively cycling.⁹ In addition, by interrogating the stem cell capacity and cell cycle status of distinct subpopulations of marrow cells typically discarded during conventional stem cell isolation strategies, we have found that both the lineage-positive and lineage-negative cellular fractions contain actively cycling stem cells and that these cycling stem cells appear to be preferentially lost in standard HSC purifications.⁹ Numerous older studies have shown fluctuations in stem cell homing, differentiation and engraftment capacity linked to cell cycle transit^{10–12} and we postulate that, if marrow stem cells are cycling, they cannot be purified to ‘homogeneity’ as they will be continually changing their phenotype as they transit the cell cycle. We are embarking on studies tracking stem cell marker fluctuation with cell cycle progression. To this end, we have found that lineage negative/stem cell marker negative cells gain c-Kit and Sca-1 surface expression in culture, and are currently testing this population for a correlation between cell cycle phase, gain of c-Kit and Sca-1 expression and stem cell function. In sum, our data indicate that stem cell purification skews isolation towards a small population of quiescent stem cells and underrepresents the cycling stem cells, perhaps in part due to cell cycle-linked fluctuations in stem cell markers. Given the loss of a large pool of actively cycling HSCs during standard stem cell isolation techniques, these data underscore the need to re-evaluate the total hematopoietic stem cell potential in marrow on a population level in addition to

ongoing investigations of purified stem cells in order to provide a more comprehensive study of HSC biology.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

LG received grant support from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health through Grant Number P20GM103468 and from the Herbert W. Savit Research Fellowship. The symposium and publication of this supplement were sponsored by the Division of Hematology/Oncology at the Warren Alpert Medical School of Brown University and NIH Center of Biomedical Research Excellence (COBRE) for Stem Cells Biology at Rhode Island Hospital.

REFERENCES

- Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med* 1996; **183**: 1797–1806.
- Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1994; **1**: 661–673.
- Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 1996; **273**: 242–252.
- Christensen JL, Weissman IL. Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc Natl Acad Sci USA* 2001; **98**: 14541–14546.
- Oguro H, Ding L, Morrison SJ. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. *Cell Stem Cell* 2013; **13**: 102–116.
- Fleming WH, Alpern EJ, Uchida N, Ikuta K, Sprangrude GJ, Weissman IL *et al.* Functional heterogeneity is associated with the cell cycle status of murine hematopoietic stem cells. *J Cell Biol* 1993; **122**: 897–902.
- Passegué E, Wagers AJ, Giurato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. *J Exp Med* 2005; **202**: 1599–1611.
- Orschell-Traycoff CM, Hiatt K, Dagher RN, Rice S, Yoder MC, Srour EF *et al.* Homing and engraftment potential of Sca-1⁺lin[−] cells fractionated on the basis of adhesion molecule expression and position in cell cycle. *Blood* 2000; **96**: 1380–1387.
- Goldberg LR, Dooner MS, Johnson K, Papa E, Pereira M, Del Tatto M *et al.* The murine long-term multi-lineage renewal marrow stem cell is a cycling cell. *Leukemia* 2014; **28**: 813–822.
- Lambert J-F, Liu M, Colvin GA, Dooner M, McAuliffe CI, Becker PS *et al.* Marrow stem cells shift gene expression and engraftment phenotype with cell cycle transit. *J Exp Med* 2003; **197**: 1563–1572.

11 Colvin GA, Dooner MS, Dooner G, Sanchez-Guijo FM, Demers DA, Abedi M *et al.* Stem cell continuum: directed differentiation hotspots. *Exp Hematol* 2007; **35**: 96–107.

12 Cerny J, Dooner M, McAuliffe C, Habibian H, Stencil K, Berrios V *et al.* Homing of purified murine lymphohematopoietic stem cells: a cytokine-induced defect. *J Hematother Stem Cell Res* 2002; **11**: 913–922.