



SPOTLIGHT ON HEMATOPOIETIC STEM CELLS: LOOKING BEYOND DOGMA

Introduction

JA Nolte and CT Jordan

Children's Hospital of Los Angeles, Los Angeles, CA, USA

Some of the long-established dogma concerning normal hematopoietic and leukemic stem cell phenotype and function have been questioned in the past few years. As a result, recent publications have been exciting and presentations at Hematology meetings have sometimes been highly controversial. This section will address some of the new cutting-edge experimentation that is going on in the field, with special attention to novel, well-documented evidence that alters our perception of old stem cell dogma.

An initial topic that has been recently reviewed in this area is that evidence now exists to support the presence of both murine and human hematopoietic stem cells that lack expression of the CD34 marker (reviewed in Refs 1–3). There appears to be a degree of reversibility of expression of CD34 at the stem cell surface, in both murine⁴ and human stem cells.⁵ The appearance and disappearance of the cell surface protein could complicate CD34⁺ cell immunosolation methods that are designed to debulk or T cell deplete bone marrow or peripheral blood products for transplantation. However, the reversibility data suggests that the CD34⁺ stem cell pool may be able to generate the CD34⁻ pool, and vice versa. Whether the two pools have different generative potentials, either in the number or in the type of progeny that they produce, remains to be determined.

One of the initial reviews for the 'beyond dogma' section, in this issue, is by Dr Mick Bhatia's laboratory and suggests that an earlier stem cell marker than CD34 may be the cell surface molecule CD133, recognized by the AC133 antibody produced by Miltenyi Biotec (Auburn, CA, USA). Dr Bhatia's laboratory has demonstrated that the AC133⁺ CD34⁻ lin⁻ population isolated from human mobilized peripheral blood can generate CD34⁺ progenitors. These data are discussed in his review in this issue. Further reviews and primary data on the phenotype and function of primitive stem cells, and on the regulation of expression of CD34 and other stem cell surface markers will be solicited for the 'Beyond Dogma' section.

Other data to be presented in this Spotlight section will concern the reversibility of not only CD34 but also of CD38 and HLA-DR, molecules that have been used as a basis for isolation procedures, and that are now found to change in culture without apparent alterations in function to accompany the change in phenotype. These data make the reisolation of cultured stem cells, such as those defined by the CD34⁺/CD38⁻ phenotype, highly unreliable.⁶ The dissociation of phenotype and function, as Dr John Dick has described, is something to be carefully considered with any population of stem cells that has been identified primarily by cell surface markers. Many laboratories are now simply using lineage-

depleted human stem cell populations in their experiments, until further knowledge is gained that will allow more accurate isolation.

An alternative method to isolate primitive stem cells that is rapidly gaining popularity, is based upon the enhanced capacity of the most primitive stem cell populations to efflux Hoechst dyes, as has been described by the groups headed by M Goodell and R Mulligan.^{7–9} This strategy is based more on function than on phenotype. Dr Goodell will write a review for the 'Beyond dogma' section on the Hoechst dye-excluding 'side population', or 'SP' cells that her laboratory has characterized. Similarly, Dr Brian Sorrentino's laboratory has determined that the *Bcrp1*/ABCG2 molecule is responsible for effluxing the Hoechst dyes from murine and human hematopoietic cells, and may be a novel hematopoietic stem cell marker. Of interest, Hoechst dye-excluding SP populations can be detected in multiple tissues, including muscle, brain, liver and kidney, in addition to bone marrow. This population is thus important in studies of so-called 'stem cell plasticity' discussed briefly below.

Rodent transplantation studies have suggested the existence of totipotent stem cells in the bone marrow compartment, capable of generating not only blood cells, but also liver or muscle. Lagasse *et al*,¹⁰ in Markus Grompe's group reported that c-kit⁺ Thy-1.1 (lo) Lin⁻ sca-1⁺ stem cells, isolated from the bone marrow of adult mice, rescued the liver defect in the FAH^{-/-} mouse, an animal model of tyrosinemia type I, by restoring biochemical function to the liver. The same population of cells was also capable of radioprotection of the lethally irradiated mice, in the same study.¹⁰ The c-kit⁺ Thy-1.1 (lo) Lin⁻ sca-1⁺ (KTLS) population of murine stem cells had been shown to be radioprotective,^{11–13} but the elegant demonstration by Lagasse *et al* that the same population could also generate functional hepatocytes was highly novel. There may have been one single cell type that was responsible, a primitive totipotent cell, or there may be two separate cells with liver and blood regenerating capacity within the stem cell population that was used. The different theories of plasticity are shown in Figure 1.

Jackson *et al*¹⁴ demonstrated that a portion of the Hoechst dye-excluding SP cells isolated from the skeletal muscle of adult mice were capable of hematopoietic differentiation. In another study, murine SP cells isolated from bone marrow were found to assimilate into the muscle of a dystrophin-deficient mouse after systemic infusion.¹⁵ In a third mouse model of stem cell plasticity, c-kit⁺/lineage⁻ cells isolated from rodent bone marrow had the capacity to heal cardiac infarcts.¹⁶ These data demonstrate that cells capable of generating blood can be found in unexpected places, and that marrow-derived cells can display potentials that were not previously thought to exist in the marrow compartment. These revolutionary studies therefore challenge the old dogma that bone marrow stem cells exist only to make blood.

There may be uncommitted, totipotent cells within the bone

Correspondence: JA Nolte, Children's Hospital of Los Angeles, 4650 Sunset Blvd, Mailstop 62, Los Angeles, CA 90027, USA; Fax: 323 660 8736

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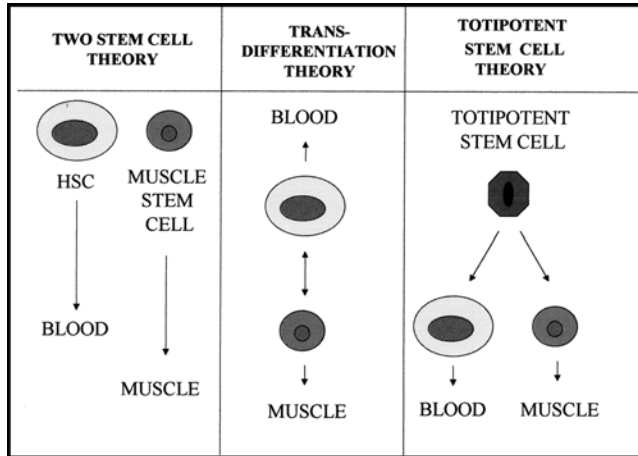


Figure 1 Stem cell theories: Left panel: Two stem cells with different fates reside in the same location and are isolated together, giving rise to two tissues in subsequent assays. Central panel: Stem cells restricted to one fate can trans-differentiate to a different tissue, dependent on the microenvironment or other cues. Right panel: One totipotent, or uncommitted stem cell can generate two different tissues.

marrow that can generate both blood and other tissues such as muscle or liver. An alternative explanation for the observed 'plasticity' of marrow-derived stem cells could be that liver or muscle-committed cells reside in the bone marrow, in addition to the blood-forming cells (Figure 1). To discriminate between the 'totipotent stem cell' and 'two stem cell' theories, retroviral or lentiviral marking of the individual stem cells will need to be done, with tracking of the same stem cell into progeny of two disparate cell types using clonal integration analysis (Figure 2), as our group has previously described in the transduction of human hematopoietic stem cells that were primitive enough to generate both T lymphoid and myeloid progeny.¹⁷

There are few data indicating that human counterparts may exist for the murine and rat stem cells that display plasticity,

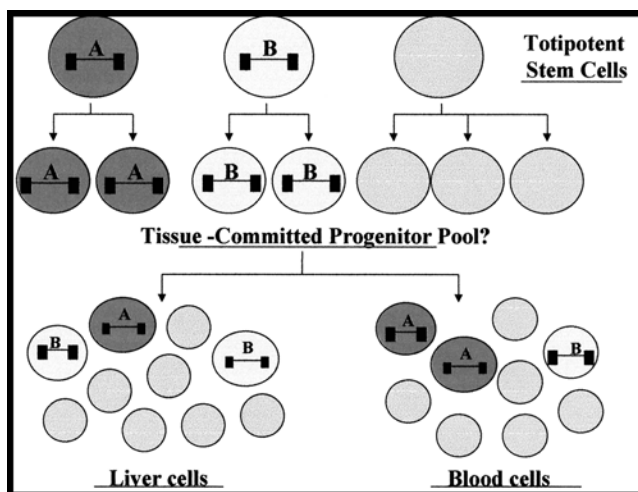


Figure 2 Clonal integration analysis to track tissue origin. One definitive way to determine that cells from two disparate tissues are derived from a common stem cell is through the use of clonal integration marking. Retroviral vectors integrate randomly into the cellular DNA of the target stem cell, providing a clonotypic marker that can be identified and tracked in the progeny of the stem cells by sensitive PCR-based methods.

due to a lack, so far, of appropriate *in vivo* models for studying human stem cells that may have the capacity to generate multiple tissues. In retrospective studies, Neil Theise, Diane Krause and colleagues demonstrated that in humans, hepatocytes and cholangiocytes could be derived from bone marrow.¹⁸ They analyzed archival liver specimens from female recipients of bone marrow transplantations from male donors, and found Y chromosome-positive hepatocytes and cholangiocytes in the female BMT recipients.¹⁸ This observation suggests that marrow-derived stem cells can generate liver in humans. Drs Theise and Krause will provide a review of the very exciting work from their laboratories in the 'Beyond dogma' forum.

The possibility that a population of totipotent, uncommitted stem cells may exist in the bone marrow is intriguing, and if a human counterpart exists, it could have immense clinical benefit. We can imagine that in the future a marrow-derived stem cell transplant could be used to repair injury to a patient's liver or heart, rather than the patient needing to endure the long wait for a cadaveric donor. Totipotent, pre-hematopoietic stem cell populations may also be identified, in the future, that are free of leukemic cells, since most of the stem cell phenotypes identified to date can contain both normal and leukemic cells, as discussed below.

Another important aspect of stem cell biology that will be addressed in the 'Beyond dogma' section concerns the origins of hematologic malignancy. Recent studies have characterized stem cell populations for both acute and chronic myelogenous leukemia and have greatly enhanced our understanding of the biology of these diseases. For example, stem cells for acute myelogenous leukemia (AML) have now been phenotypically described in a fashion similar to that of normal stem cells. Studies by Dick and colleagues have shown that primary AML cells with a CD34⁺/CD38⁻ phenotype are sufficient to perpetuate leukemic growth in the xenogeneic NOD/SCID mouse model system.^{19,20} Importantly, stem cell engraftment in this model is dose-dependent and sufficient to transplant secondary recipients. Similarly, a report by Blair *et al*²¹ has shown that CD34⁺/CD71⁻/HLA-DR⁻ AML cells can engraft NOD/SCID mice and maintain long-term culture growth *in vitro*. Of interest, Blair and colleagues^{22,23} have also shown that AML stem cells lack expression of Thy-1 and CD117. These observations are important because they represent the first reports of antigenic features of primitive leukemia cells that differ from normal stem cells. More recently, further studies have shown that CD123 is differentially expressed between malignant and normal stem cell populations, with high levels of CD123 found on leukemic stem cells.²⁴ New reports of markers that could potentially be used to separate leukemic from normal stem cells are sought for the 'Beyond dogma' section.

The availability of antigenic markers that distinguish leukemic from normal stem cells has permitted more detailed molecular analyses of primitive leukemic cells. For example, two recent studies have described aberrant expression of tumor suppressor genes and the transcription factor NF- κ B in primitive AML but not normal hematopoietic cells.^{25,26} Several interesting advances have also been made for chronic myelogenous leukemia (CML), which will be reviewed in the 'New dogma' section by Connie Eaves and her colleagues. Perhaps most importantly, it has been shown that primitive CML cells appear to possess autocrine cytokine activity that may be central to their cell cycle activation and differentiation.²⁷⁻²⁹ In addition, it has also been shown that at least some CML stem cells are highly quiescent, much like their normal stem cell

counterparts.³⁰ Taken together, these studies, as well as others, are shedding new light on the nature of leukemogenesis and the properties of primitive malignant cells.

Exciting data have also been recently reported in the areas of stem cell homing after transplantation. The use of immune-deficient mouse xenograft systems to study human stem cell homing has revolutionized the field, and molecules that activate homing receptors have been identified. Dr Tsvee Lapidot will contribute a mini-review on the interesting work done in his laboratory on SDF-1 activation of the CXCR4 homing receptor, thought in previous years to be most important on T cells as the co-receptor for the HIV virus. Although somewhat controversial,³¹ the work of Dr Lapidot and others has shown that the CXCR4 receptor plays an additional role in the *in vivo* homing and engraftment of stem cells.^{32,33}

Another exciting area of research in the stem cell homing area is discussed in this issue by Ed Srour, who along with Peter Quesenberry and Pamela Becker's groups, have shown that non-cycling hematopoietic stem cells home back to the bone marrow more efficiently than cycling cells.^{34,35} This research is highly important for the field of *ex vivo* stem cell expansion and for the field of gene therapy, which primarily concentrates on inducing cells to cycle to allow integration of mitosis-dependent viral vectors. It is possible that the success of gene therapy could be significantly improved if the stem cells could be returned to the quiescent phase after viral entry and integration but prior to transplantation. Thus, although controversy exists at the moment, the issues of stem cell cycle and homing are highly important. Dr Ed Srour will discuss the controversies that currently exist in the area of hematopoietic stem cell homing.

In addition to the exciting reviews that we have planned for this section, we are soliciting suggestions for other reviews or primary data that fit with or augment the themes described above. For participation in the 'Spotlight on hematopoiesis: looking beyond dogma' section, please send ideas for reviews to jnolta@chla.usc.edu or submit completed manuscripts to the *Leukemia* Editorial Office in Paris labeled: 'Spotlight on Hematopoiesis (Beyond dogma section) for the attention of Dr JA Nolta (normal stem cells) or Dr C Jordan (leukemic stem cells)'.

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