Stem Cell Plasticity

A Rare Cell, Not a Rare Event

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Abstract

Purification to homogeneity for a rare stem cell (SC) population by both function and phenotype is a prerequisite to determine if SCs can change their fate (plasticity). Since cell fate determination has been suggested by both external environmental cues and intrinsic gene regulation, plasticity should be studied using both influences. Different frequencies of marrow SC plasticity may be attributed to either different isolation technologies or different developmental stage SCs with more or less multipotentiality. Tissue-specific SCs may reside in marrow, or alternatively, primitive marrow SC may respond directly to regenerative signals by migration to injury sites and repairing the damaged tissue. It is important to dissect the relationship between primitive/tissue-specific SCs and regenerative signals.

Index Entries: Plasticity; marrow stem cell; tissue-specific stem cell; regenerative signals; cell fate change.

Introduction

Although there have been many reports which have demonstrated the conversion of adult marrow cells into developmentally unrelated cell types (reviewed in refs. 1–4), the nature of the cells which are the source for conversion have not been clearly elucidated. Given that many earlier studies used heterogeneous marrow cell populations, it has been difficult to interpret that the cell source for conversion was of any particular cell type. Furthermore, the stem cells (SCs) isolated from one study may not have the same capacity for differentiation as those isolated from other studies. It is possible to speculate that even within the SC population(s) in the bone marrow (BM) there may be different developmental stages which represent SCs with more or less multipotentiality. Thus, purification to homogeneity for a rare SC population is a prerequisite to determine if SCs can change their fate (plasticity).

Isolation of Hematopoietic Stem Cells

The classical properties of hematopoietic stem cells (HSCs), the most primitive progenitors of all blood cells, are that HSCs have the capacity for self-renewal and for the long-term production of all blood cell lineages. HSCs have been enriched using a variety of techniques, including pharmacological manipulation (5), elutriation (6,7), staining with antibodies (8–11), and use of supravital dyes (12–15); however, few unique characteristics have been found to specifically identify these rare and elusive cells (16). Mouse HSCs isolated by cell-surface markers have been reported to be responsible for both radioprotection (rapid engraftment that will prevent early death from radiation-induced marrow aplasia) and long-term repopulation (LTR) of all blood cell lineages (8). These HSCs isolated by phenotype may be biologically heterogeneous. It is now
evident that HSCs are very slowly proliferating cells that generate delayed multilineage engraftment (LTR), while radioprotection is attributed to more committed progenitors (16–18). Although day-12 spleen colony-forming units (CFU-S) have been postulated to be primitive multipotential hematopoietic progenitors, with day-8 CFU-S representing later, more committed progenitors, neither of these CFU-S represents mouse HSC. There are two vital classes of engrafting cells: (1) committed progenitors, which provide initial, unsustained engraftment (i.e., 4–6 wk), and (2) HSC, which produces delayed, but durable engraftment (i.e., 4–6 mo and beyond). Therefore, for late hematological reconstitution, HSCs must be transplanted with a source of early engrafting cells, thereby allowing the lethally irradiated host to survive initial aplasia.

Counterflow centrifugal elutriation (CCE) can be used to separate short and long term repopulating cell populations (STRC and LTRC, respectively) (6). We showed that 1–10 HSCs (less than or equal to 0.005% of whole bone marrow) could generate all blood cell lineages for up to the lifetime of the animal (19,20). However, these cells lacked radioprotection and spleen colony-forming activity. This rare population is at least 10-fold less frequent than those reported by others (0.05–0.1%) (21–24).

Our SC isolation procedure revealed the frequency of LTRC to be as follows:

CCE sorts cells on the basis of size and density. The fraction containing the largest cells (rotor off cells [RO]) was enriched for the granulocyte-macrophage colony-forming units (CFU-GM), but gave only transient, early engraftment, and was therefore depleted of LTRC (6). The intermediate fractions (FR29 and FR33) were enriched for CFU-S, but depleted of CFU-GM. Despite being devoid of CFU-GM and CFU-S, the fraction 25 (FR25) consisting of only morphological lymphocytes gave sustained, albeit delayed, reconstitution of all hematopoietic cells, and was therefore enriched for HSC. Each of the four fractions isolated by CCE contained 10–20% of whole marrow cells (6).

AA4.1, an antigen that is expressed by most mouse hematopoietic progenitors and B cells, was also found to be expressed on HSC from mouse fetal liver (25) and 5-fluorouracil-treated adult bone marrow (5). However, we found that only the FR25 AA4.1- lin- marrow cells (0.3% of whole marrow) produced long-term engraftment (19), whereas the small AA4.1+ lin- cells were unable to produce long-term engraftment, as cells have also reported (26,27). It has been suggested that the cells in the FR25 lin- population were the most primitive set of HSCs and contains very few day-12 CFU-S obtained from adult murine bone marrow (28).

HSCs express high levels of aldehyde dehydrogenase (ALDH) (29,30), an enzyme responsible for the conversion of vitamin A to its active metabolite retinoic acid (31,32) and for cellular resistance to cyclophosphamide (33). We further enriched HSCs by isolating small AA4.1- lin- cells that highly expressed ALDH. The FR25 AA4.1- lin- ALDH+ cells represent 0.005 ± 0.001% of the whole marrow (19). This allowed us, using 8–10 mice, to recover in each experiment (1–2) × 104 of these cells. For transplantation studies, these cells were combined with a distinguishable source cells that provide radioprotection: the RO fraction that is enriched for committed progenitors while depleted of HSC. When transplanted into marrow-ablated female mice with 2 × 104 female RO marrow cells, 10 male FR25 AA4.1- lin- ALDH+ marrow cells were able to generate male peripheral blood cells present in the mice for up to 15 mo.

In contrast, mouse HSCs isolated by surface marker phenotype alone (approximately at a frequency of 0.04–0.09%) are confined to the Thy-1.1(lo) lin(−/−) Sca-1+ fraction of bone marrow (21–23). Using CCE the peak number of Thy-1.1(lo) lin(−/−) Sca-1+ cells was highly enriched in one eluted fraction (FR29), which was also highly enriched for day-12 to -13 CFU-S (21). However, FR25 Thy-1.1(lo) lin(−/−) Sca-1+ cells were relatively depleted of CFU-S 12 to 13.

Taking together all of these studies, it appears that the FR25 lin- population represents a class of SCs which is less frequent, smaller sized, and contains only LTR capacity of the hematopoietic organs devoid of STRC responsible for radioprotection (CFU-S 12).

LTRCs remain quiescent in the bone marrow shortly after engraftment, whereas STRCs which radioprotect are more rapidly dividing (19,34). We utilized a dye, PKH26, which incorporates into the membrane of cells and is equally distributed to daughter cells when they divide. We were able to retrieve PKH26 labeled FR25 AA4.1- lin- cells post-transplant in the hematopoietic tissues of the recipients. We demonstrated the FR25 AA4.1- lin- cells homing to bone marrow remain quiescent and could long term engraft mice. Even though cells are homed both the spleen and bone marrow, the cells in the bone marrow were significantly more competent at reconstituting lethally irradiated secondary hosts (34).

We have extended limiting dilution studies from 10 cells to the single-cell level. We demonstrated that a single bone marrow PKH26+ FR25 AA4.1- lin- cell selected in vivo by homing (2 d in a second recipient) to marrow was able to long-term repopulate both hematopoietic and nonhematopoietic tissues and self-renew by serial transplantation (20,35). The variable levels of donor engraftment in peripheral blood and bone marrow of the recipients with a single pure HSC revealed the expansion, differentiation, and exhaustion of a single SC in vivo. In addition, these cells were found to express undetectable or low levels of many surface antigens, such as Thy-1, c-Kit, Sca-1, and CD34, presumed to mark early hematopoietic cells (19,20). We have demonstrated that the HSC compartment is phenotypically heterogeneous with populations of HSC that are positive and negative for CD34 expression (36). It may be that expression of CD34 is related to cell cycle activation (37) and may be reversible in vitro (38). Marrow homing was used to further enrich HSC without using specific surface markers for positive selection (20). During the homing, CD34 and SCA-1 expression increases uniquely on cells that home to marrow. LTR of irradiated hosts and serial transplantation to secondary hosts indicates the self-renewal capacity of 2 d homed PKH+ FR25 AA4.1- lin- cell.

Epithelial Tissue Engraftment by Stem Cells

LTR of irradiated hosts and serial transplantation to secondary hosts represent the gold standard for demonstrating self-renewal and differentiation, the defining properties of HSC. A 2d homed PKH+ FR25 AA4.1- lin- cell has tremendous differentiative capacity as it can also differentiate into epithelial cells of the lung (bronchi and alveoli), gastrointestinal tract (esophagus, stomach, small bowel, and colon), renal tubules,