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Further Proof for an Unpopular Concept: A Single Cell From Bone Marrow Can Serve as a Stem Cell for Both Hematopoiesis and Osteogenesis

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In science, unpopular concepts must be proven time and again. The article by Hofmann *et al.* in this issue of *Molecular Therapy* provides further proof for the unpopular idea that a single cell from bone marrow can serve as a stem cell for both hematopoiesis and osteogenesis.[1] Moreover, Hofmann *et al.* establish that this single cell is actually an old friend: the long-term repopulating hematopoietic stem cell (LTR-HSC). These observations also suggest, but do not conclusively prove, the interesting idea that the same LTR-HSC can provide both the HSC and the microenvironmental niche required for the HSC to self-replicate and differentiate. Such a dual function can easily explain why an LTR-HSC is a long-term repopulating cell. The study provides new evidence for a refreshingly simple concept that probably should have occurred to us earlier.

The story that Hofmann *et al.* tell has a long and tangled history. The early contributions of many scientists to this history can be readily accessed only through the titles of their publications because of the limited retrospective reach of PubMed.[2–4] The contributions of others have faded because of artifacts that were either not recognized at the time or as yet undiscovered. At any rate, early research on hematopoiesis clearly demonstrated a close relationship to osteogenesis. In addition, there were suggestions that both hematopoietic cells and bone cells arose from the same cell. These suggestions were based on many observations, including one from embryology that showed that bone marrow and endochondrial bone arose from the same vascular cells that invaded the initial cartilage molds of long bones. But these observations were difficult to integrate into the paradigm that quickly dominated the field, namely, that blood and bone arose from two distinct classes of stem cells.

Specifically, the work of Hofmann *et al.* began more than 10 years ago with earlier work by the senior author of the paper, Edwin Horwitz.[5,6] Horwitz and colleagues carried out a pioneering clinical trial with bone marrow cells in children with osteogenesis imperfecta (OI, also known as brittle bone disease). The trial was based on observations in my own laboratory with transgenic mice that developed a phenotype resembling OI because they expressed a mutated human gene for type I collagen that was discovered in a patient with OI.[7] The mice improved after being marrow ablated and then receiving infusions, from normal mice, of both bone marrow and genetically marked plastic-adherent cells from bone marrow that are referred to as fibroblastic colony-forming units, mesenchymal stem cells, or marrow stromal cells (MSCs).[8–12]

On the basis of these observations, Horwitz and associates[5,6] treated several children with severe OI first with a marrow transfusion from normal donors and then several years later with MSCs from the same or haplotype-matched donors. The children were not cured but showed temporary improvement in several clinical parameters. Importantly, the children exhibited no serious adverse effects. These observations, together with a subsequent observation by Le Blanc *et al.*,[13] who administered MSCs to a child with severe graft-versus-host disease, opened a floodgate of clinical trials with MSCs in a wide variety of diseases. More than 200 trials with MSCs or similar cells have been registered, and some have reached phase II or III (ClinicalTrials.gov).

But the story took a strange and unexpected turn. Horwitz and his colleagues attempted to demonstrate engraftment of MSCs in bones of mice. To their surprise, they found that a fraction of nonadherent cells from bone marrow that contained HSCs engrafted into bone far more efficiently than plastic-adherent MSCs.[14] They published this observation more than 10 years ago. Their results were consistent with previous reports by several groups of investigators. For example, Nilsson *et al.*[15] observed that whole bone marrow could generate bone cells in nonablated mice. Olmsted-Davis *et al.*[16] found that a single adult hematopoietic cell ("side population" cell) could function as an osteoblast in mice. The publications were largely ignored by most investigators in the field, including me. Why? There were two reasons. One was that demonstrating engraftment of cells into bone is technically difficult. Sectioning of bone for microscopy requires decalcification, during which much of the cellular architecture is lost. Also, the tissue has a high degree of autofluorescence. Extracting DNA or RNA from bone is equally difficult. In addition, it is impossible to obtain quantitative data on engraftment into the whole skeleton. Therefore, the doubters in the field raised technical objections, some of which were valid.

But a more compelling reason for ignoring these findings is that the results contradicted the prevailing paradigm in the field that marrow contained two distinct classes of stem cells: HSCs and MSCs. The paradigm was based on several well-documented observations, for example, that viable cultures of HSCs[17] required feeder layers of fibroblasts or the plastic-adherent and fibroblast-like MSCs from bone initially identified by Owen and Friedenstein.[8] The paradigm had tremendous appeal because it was simple and it invited research on the

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two kinds of stem cell. Research on MSCs was particularly appealing, because these cells could be expanded in culture far more easily than HSCs. And the research paradigm was highly productive. However, the excitement about HSCs and MSCs as separate classes of stem cells pushed aside any thought of searching for a common precursor cell, or even a cell that engrafted in bone more efficiently than MSCs.

Despite the reception to their initial publications, Horwitz *et al.* and several other research groups[18–20] persevered. In their paper in this issue, Hofmann *et al.* provide some very convincing data based largely on experiments with bone marrow and marrow cells from transgenic mice ubiquitously expressing green fluorescent protein (GFP).[1] First they confirmed that $Lin^{-}Sca1^{+}$ and $cKit^{+}Lin^{-}Sca1^{+}$ cells (KLS cells) from these mice generated GFP-labeled osteoblasts and osteocytes after administration to lethally irradiated mice of the same strain. $Lin^{-}Sca1^{-}$ cells reconstituted short-term, but not long-term, hematopoiesis and did not give rise to osteoblasts.

The authors further defined the phenotype of the osteogenic cells as $CD34^{-}KLS$ cells, the phenotype usually ascribed to LTR-HSCs. In a critical experiment, they demonstrated that just 300 unlabeled $CD34^{-}KLS$ cells competitively inhibited the engraftment into bone cells observed with unfractionated bone marrow cells from the GFP mice. Then they performed an ultimate test of stem cells: they injected a single GFP-labeled KLS cell (along with unlabeled $Sca1^{-}$ cells) into each of a large number of irradiated mice ($n = 60$). Four of the mice demonstrated significant engraftment of GFP-expressing cells into multiple tissues. Bone marrow from these mice was transplanted into secondary recipients. GFP-labeled osteoblasts and osteocytes were again found in several of the secondary recipients. The results therefore present a convincing case that cells previously shown to be LTR-HSCs are also stem cells for osteoblasts. In addition, the authors observed that the labeled osteoblasts and osteocytes in the secondary recipients were found in clusters similar to clusters of HSCs seen after marrow transplants. Therefore, the GFP-expressing osteoblasts could have contributed to a niche for the HSCs.

The data presented by Hofmann *et al.*, as well as similar observations by others, may show that we have moved too fast. We may have walked past a simple biological truth that unifies our thinking about bone marrow and stem cells in general. Embarrassingly for us all, it is a truth that was apparent from the earliest observations of stem cells in biology: stem cells do not exist in isolation. They need nurse cells or feeder cells that provide the niche to support their self-renewal and differentiation. The importance of the stem cell niche is dramatically illustrated in simple systems such as germline stem cells in *Drosophila*.[21] The lesson was largely overlooked in research on hematopoiesis, in which it was generally assumed that HSCs were preprogrammed to differentiate via a hierarchical pattern that required prodding from only a few cytokines to define the fate of the progeny. As Orkin and Zon[22] concluded several years ago after an exhaustive review of hematopoiesis, "The 'classical' hierarchy diagram…provides a seductive, but overly simplified view."

The importance of a niche for HSCs in marrow is now generally recognized, and defects in that niche may underlie many diseases.[23,24] The nature of the niche is still poorly understood, but it includes MSC-like cells and cells of the osteolineage.[25,26] If LTR-HSCs can provide both the stem cells for hematopoiesis and the cells that are required for the niche, it is no surprise that they are long-term repopulating cells. And the potential of LTR-HSCs to generate their own niches is reminiscent of the tendency of MSCs to generate their own microenvironments as they are plated at clonal densities so that colonies develop from single cells.[27]

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