Hematopoietic stem cell origin of mesenchymal cells: opportunity for novel therapeutic approaches

Makio Ogawa · Amanda C. LaRue · Patricia M. Watson · Dennis K. Watson

Abstract There has been a general belief that there are two types of adult stem cells, i.e., hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), each with distinctly different functions. According to this dogma, HSCs produce blood cells, while MSCs are thought to generate a number of non-hematopoietic cells including fibroblasts, adipocytes, chondrocytes and bone cells. Recently, a number of studies, including those in our laboratory based on single HSC transplantation, blurred the clear distinction between HSCs and MSCs and strongly suggested an HSC origin of the adult mesenchymal tissues. This review summarizes the experimental evidence for this new paradigm and the literature pointing out the vagary in the stem cell nature of MSCs. The concept of the HSC origin of mesenchymal cells will have many immediate and long-term impacts on the therapies of diseases and injuries of the connective tissues.

Keywords Hematopoietic stem cells · Mesenchymal cells · Origin · Transplantation · Connective tissues · Adipocytes · Fibroblasts · Bone cells · Chondrocytes

1 Introduction

It is generally believed that there are two types of stem cells in the bone marrow, i.e., hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), and that their repertoire of differentiation/reconstituting potentials are distinct and separate from each other. HSCs have been shown to produce blood cells and some cells in the tissues such as mast cells and osteoclasts. MSCs are thought to generate a number of non-hematopoietic cells including fibroblasts, adipocytes, chondrocytes and bone cells (i.e., osteoblasts and the terminally differentiated osteocytes) and to support proliferation and differentiation of HSCs and their progenies [1–6]. Mesenchyme, or mesenchymal connective tissue, represents the part of the embryonic mesoderm that consists of loosely packed, non-specialized cells embedded in a gelatinous ground substance, from which connective tissues and hematolymphatic systems develop. The term “mesenchymal stem cells” was coined by Caplan [7] in 1991 to describe a population of cells in the adult bone marrow that can be isolated, expanded in culture and stimulated to differentiate into bone, cartilage, muscle, marrow stroma, tendon, fat and a variety of other connective tissues. Clarification of the lineage potentials of MSCs by Friedenstein and his associates, however, had begun prior to Caplan’s report. In their classic series of studies, they demonstrated that fibroblasts and fibroblast colony-forming units (CFU-F) [8, 9] are the precursors of bone cells and chondrocytes [10]. Because large numbers
of MSCs can be generated in culture, MSCs have been thought to be useful for "tissue-engineering" of the connective tissues [11].

2 Single HSC transplantation

In contrast to this popular belief, studies in our laboratory based on single HSC transplantation strongly indicate that multiple connective tissues are generated by HSCs. Several years ago, during the height of stem cell plasticity controversies, we began single HSC transplantation to obtain unequivocal conclusions on the tissue-reconstituting capability of HSCs. We used the transgenic enhanced green fluorescent protein (EGFP) mice [12] and CD 45.1 C57BL/6 mice as the donor and the lethally irradiated recipient mice, respectively. We reasoned that, to study the full differentiation potentials of HSCs, it is necessary to generate mice exhibiting high-level, multi-lineage engraftment from single HSCs. In our hands, however, direct transplantation of single bone marrow cells with lineage (Lin)− Sca-1+ c-kit+ CD34− cells or Lin− Sca-1+ CD34− side population (SP) cells [16] from EGFP mice were individually deposited into the wells of 96-well tissue culture plates and, after confirmation of the presence of a single cell per well, cultured for 1 week in the presence of stem cell factor and interleukin-11 (IL-11) or a combination of steel factor and granulocyte colony-stimulating factor (G-CSF). Earlier, we had observed that both IL-11 [17] and G-CSF [18] act on cell cycle dormant primitive multi-potential progenitors and induce cell divisions. Because the majority of HSCs are dormant in cell cycle and do not begin cell division until a few days after initiation of cell culture, transplantation of clones consisting of 20 or fewer cells after 1-week incubation significantly raised the efficiency of generating mice with high-level multi-lineage engraftment [14, 15].

Two months to 1 year after cell transplantation, nucleated blood cells from these mice were analyzed for hematopoietic engraftment and only the mice revealing high-level, multi-lineage engraftment by donor EGFP+ cells were selected for analysis of tissue reconstitution. We also carried out transplantation of 100 un-cultured Lin−, Sca-1+, c-kit+, CD34− cells and made similar observations to those seen in clonally engrafted mice. The latter finding excluded the possibility of cell fusions by carrying out male-to-male or female-to-male transplantation and analyzing the number of Y-chromosomes in the EGFP+ cells. In the subsequent series of investigations, we discovered that several types of fibroblasts/myofibroblasts are derived from HSCs via non-fusion mechanisms as summarized in a recent review [19].

3 HSC origin of fibroblasts/myofibroblasts

Tissue fibroblasts play a key role in growth factor secretion, matrix deposition and matrix degradation and, therefore, are important in many pathological processes. For example, fibroblasts are critical to the inflammatory response and its control at the time of tissue injury. Fibroblasts participate in wound healing by producing extracellular matrix proteins, responding to and synthesizing cytokines, chemokines and other mediators of inflammation (for reviews, see [20, 21]). In addition, fibroblasts can be activated to become myofibroblasts, which being armed with myosin and alpha smooth muscle actin, exert contractile force to reduce the size of the wound. Uncontrolled proliferation and/or activation of these cells result in tissue fibrosis. Fibroblasts and myofibroblasts are also important in the steady state physiology of many organs and tissues. In general, they confer the structural integrity of the tissues and support the proliferation and differentiation of other cell classes such as epithelial cells. A number of myofibroblasts with defined tissue-specific functions have also been described. For example, contractile myofibroblasts, such as glomerular mesangial cells in the kidney, hepatic stellate cells and pericytes function as regulators of blood flow. An example of even more specialized myofibroblasts is the interstitial cells of Cajal in the intestines that control intestinal motility. Readers are referred to reviews [22, 23] for a list of myofibroblasts and their functions.

The first type of myofibroblasts we detected in the mice engrafted with single HSC was glomerular mesangial cells of the kidney [14]. The location in the kidney and morphology of the EGFP+ cells suggested that the cells were mesangial cells. Their identification was confirmed by the ability of the EGFP+ cells to contract on exposure to angiotensin II. Next, we discovered that brain microglial cells and perivascular cells are of HSC origin and observed that induction of stroke by ligation of middle cerebral artery enhances the recruitment of the EGFP+ microglial cells to the injury site [15]. Morphological and immunohistochemical properties of the perivascular cells were consistent with the cells being pericytes rather than endothelial cells. We then identified HSC-derived fibroblasts associated with transplantable murine melanoma and Lewis lung carcinoma [24]. The EGFP+ HSC-derived cells
were demonstrated to be fibroblasts by their distinct morphology and expression of procollagen 1α1 mRNA. In addition, a subpopulation of the EGFP⁺ fibroblasts expressed α smooth muscle actin (αSMA), indicating that they were myofibroblasts. Again, prevalent in the specimens were the EGFP⁺ pericyte-like perivascular cells present on the tumor vasculature and admixed with tumor cells. Similar to our findings in the brain, simultaneous staining for CD31 expression clearly established that the EGFP⁺ cells were indeed perivascular and not endothelial cells. In other studies, we found that fibrocytes and other unidentified mesenchymal-type cells in the spiral ligament of the inner ear are of HSC origin [25]. Inner ear fibrocytes are known to play critical roles in the homeostasis of inner ear ion and fluid channels and are important for the health of the inner ear hair cells. These fibrocytes are classified into five types based on location, morphology and histochemical properties. EGFP⁺ cells were seen among all five types of fibrocytes [25]. We also discovered that fibroblasts/myofibroblasts in the adult heart valves are also derived from HSCs [26]. Investigators in other laboratories have also described the HSC origin of fibroblasts/myofibroblasts in other tissues using single HSC transplantation. It was shown that fibroblasts/myofibroblasts are traced to the site of myocardial infarction [27] and liver stellate cells, a type of specialized myofibroblasts, are derived from HSCs [28].

In addition to these in vivo studies demonstrating an HSC origin of tissue fibroblasts/myofibroblasts, we have also succeeded in the culture of fibroblasts from EGFP⁺ bone marrow cells of mice that were clonally engrafted by a single HSC [29]. EGFP⁺ bone marrow cells from these mice, incubated in fibronectin-coated tissue culture dishes or flasks in the presence of 10% mouse serum and 10% fetal bovine serum (FBS), generated adherent cells exhibiting the morphology of fibroblasts described three decades ago by Friedenstein et al. [8, 30]. The EGFP⁺ cells had prominent clear nuclei and spindle-shaped or pleomorphic cytoplasm. They also expressed mRNAs for procollagen 1α1, fibronectin, vimentin and discoidin domain receptor type 2 (DDR2). Time course flow cytometric analyses of the cultured EGFP⁺ bone marrow cells revealed gradual expression of collagen I and DDR2 and concomitant loss of CD45 during 3 weeks of incubation. During this transition, the cells at intermediate stages were CD45⁺ collagen I⁺ and DDR2⁺ and probably represent the cell culture equivalent of tissue fibrocytes to be described later. Precursors of fibroblasts (colony-forming unit fibroblasts, CFU-F) [8, 9], were also detected in the bone marrow of the clonally engrafted mice [29]. Earlier, Penn et al. [31] described that a population of cells in the murine CFU-F-derived colonies was positive for Mac-1 and F4/80. Together, these findings in cell culture were consistent with the results of transplantation studies described above and strongly suggested that most, if not all, fibroblasts/myofibroblasts are derived from HSCs.

4 HSC origin of adipocytes

Adipose tissues, scattered throughout many organs, play a critical role in energy balance. When excess calories are available, the adipose tissues grow larger via increase in both the size and number of adipocytes. Since mature adipocytes cannot divide, hyperplasia is achieved by recruitment of preadipocytes and differentiation of uncommitted precursors into the adipocytic lineage. It has been generally believed that preadipocytes are derived from MSCs in the bone marrow [2, 3]. Regarding the pathway of adipocytic differentiation from MSCs, much evidence has suggested that fibroblasts or “fibroblastic” cells are the intermediate between MSCs and preadipocytes (see reviews [32, 33]). Two-way conversion between human adipocytes and fibroblasts has been documented in culture [34]. These findings strongly suggested that adipocytes are closely related to, if not derived from, fibroblasts. Because our studies of mouse fibroblasts/myofibroblasts strongly suggested that fibroblasts/myofibroblasts are derived from HSCs, we next tested the hypothesis that adipocytes are also derived from HSCs using transplantation of single EGFP⁺ HSCs and primary culture [35]. Adipose tissues harvested from clonally engrafted mice showed EGFP⁺ adipocytes that stained positive for leptin, perilipin and fatty acid-binding protein 4. A diet containing rosiglitazone, a peroxisome proliferator-activated receptor-γ agonist, significantly enhanced the number of the EGFP⁺ adipocytes. When EGFP⁺ bone marrow cells from clonally engrafted mice were cultured under adipogenic conditions, all of the cultured cells stained positive with oil red O and sudan black B and exhibited the presence of abundant mRNA for adipocyte markers. Finally, clonal culture and sorting based studies of Mac-1 expression of hematopoietic progenitors suggested that adipocytes were derived from HSCs via progenitors for monocyes/macrophages. Pertinent to our findings are conflicting results from two bone marrow transplantation studies. First, Crossno et al. [36] reported that transplanted BM cells generate new adipocytes and that both a high fat diet and administration of rosiglitazone induce hyperplasia of EGFP⁺ adipocytes. A year later, Koh et al. [37] refuted this observation and concluded that what appeared to be adipocytes in the adipose tissues were macrophages. Our observations based on single HSC transplantation [35] supported Crossno’s conclusion and further extended their studies to identify the bone marrow progenitor of the adipocytes, the HSC.
5 HSC origin of osteo-chondrocytes

There are a number of studies suggesting an inverse close relationship between adipogenesis and osteogenesis [38, 39]. Clinically, increased adipose mass in the bone marrow is associated with primary osteoporosis [40]. Studies of cell lines in culture also demonstrated reciprocal regulation of adipogenesis and osteogenesis [41, 42]. While molecular mechanisms regulating the differentiation of adipocytes and osteocytes are being elucidated [38, 39], one important regulator of this relationship appears to be macrophage colony-stimulating factor (M-CSF). Over-expression of M-CSF increases adipose mass [43] and deficiency of M-CSF [44] or its receptor [45] is associated with osteopetrosis. Postnatal administration of neutralizing anti-M-CSF antibody induces osteopetrosis and decreases adipocyte size [46]. This inverse, but close, relation between adipogenesis and osteogenesis indicated the possibility that osteogenesis also is a function of HSCs. In my presentation at the 71st Annual Meeting of Japanese Society of Hematology, Kyoto, 23–25 October 2009, I described our preliminary findings of HSC-derived osteo-chondrocytes. Our observations are in agreement with earlier reports also proposing HSC origin of osteocytes. For example, transplantation of 3,000 side population (SP) cells that are highly enriched for HSCs generated osteoblasts in vivo [47]. Dominici et al. [48] transplanted marrow cells that had been transduced with GFP-expressing retroviral vector and observed a common retroviral integration site in clonogenic hematopoietic cells and osteoprogenitors from each of the recipient mice. These observations, together with our findings, strongly suggest that osteocytes are derived from HSCs.

6 Relationship between HSCs and MSCs

The premise that fibroblasts/myofibroblasts, adipocytes, chondrocytes and bone cells are derived from HSCs directly challenges the long held belief that these lineages originate in MSCs. MSCs, however, are poorly defined and their physiology remains virtually unknown despite significant current academic and commercial interest in their therapeutic applications. An overwhelming majority of the studies of MSCs have been carried out in culture and most in vivo experiments are site-directed transplantation experiments. Experiments based on systemic transplantation showed primarily cell lodging in the lungs [49] and low-level engraftment at the site of injury [50]. In an excellent review in 2004, Javazon et al. [51] summarized “characteristics of MSCs differ among laboratories and species, and there is no specific marker or combination of markers that identify MSCs either in vivo or in vitro. In addition, there are no quantitative assays to assess the presence of MSCs in any given population. Therefore, MSCs are currently defined by a combination of physical, morphologic, phenotypic, and functional properties, many of which are clearly non-physiologic”. These points were reiterated in a more recent review [52]. Reflecting this lack of evidence for the stem cell nature of MSCs, a position paper [53] was published by the International Society for Cellular Therapy that the word “MSCs” represents multipotent mesenchymal stromal cells. We have grown fibroblasts from the bone marrow cells of a mouse engrafted with a single HSC [29] and could induce adipocytic differentiation in all cultured fibroblasts [35]. Investigators in other laboratories have noticed molecular and functional similarities between fibroblasts and MSCs [54, 55]. Together, these findings strongly suggest that MSCs are very similar, if not identical, to the fibroblasts that are derived from HSCs. If so, it would explain the paucity of evidence for their stem cell nature and the fact that site-directed transplantation yields better tissue reconstitution than systemic infusion of MSCs.

7 Differentiation of HSCs to mesenchymal lineages

The mechanisms of mesenchymal differentiation from HSCs are not known. It is necessary to clarify first whether there are common mesenchymal precursors through which individual mesenchymal (e.g., fibroblast, adipocyte, osteocyte and chondrocyte) lineages differentiate or if individual mesenchymal lineages are derived directly from HSCs independently from each other. For both models, the next question to be elucidated is whether the commitment process is intrinsic to the cells or controlled by external factors. If intrinsic, differentiation may be fixed or stochastic (random) processes or a combination of the two mechanisms. Regardless of the mechanisms of differentiation, there are studies to suggest a close relationship between monocyte/macrophage lineage and fibroblasts and/or MSCs. It was shown that a population of cells in the murine CFU-F-derived colonies was positive for Mac-1 and F4/80 [31]. Human bone marrow mesenchymal progenitor cells that are capable of adipogenic, osteogenic and chondrogenic differentiation in culture were shown to express CD13 [56], a marker associated with granulocytes and monocytes. Human peripheral blood cells that express CD14, a surface protein preferentially expressed on monocytes and macrophages, were shown to generate multiple mesenchymal lineages including osteoblasts, adipocytes, chondrocytes and myocytes in culture [57]. In our laboratory, we examined the correlation between EGFP+ glomerular mesangial cells and expression of B cells, T cells or Mac-1/Gr-1+ cells in clonally engrafted mice. EGFP+ mesangial cells were detected only in the mice
expressing Mac-1/Gr-1+ cells in the blood [58]. Finally, the most direct demonstration of the close relationship is our single progenitor cell culture and subsequent cohort analysis for hematopoietic and adipogenic differentiation [35]. Here, bone marrow cells that are highly enriched for hematopoietic progenitors were deposited individually into round-bottomed 96-well culture plates containing a combination of hematopoietic cytokines permissive of all lineage expression and incubated for one week. Resulting clones were individually divided into two aliquots and cultured under two different conditions: One aliquot was cultured in 12-well non-tissue culture plates containing the combination of cytokines for hematopoietic growth and differentiation and the other in 12-well tissue culture plates for study of fibroblast growth. The aliquots cultured for hematopoietic growth for 10–12 days were individually deposited onto slides and stained with May-Grünwald Giemsa. When the aliquots cultured for fibroblasts reached 80% confluency, they were individually processed for adipogenesis. Correlative analyses of the cohorts for adipocytic and hematopoietic lineage expression revealed a strong connection between monocyte/macrophage lineage and adipogenesis [35].

Transition of hematopoietic cells to adipocytes/fibroblasts is a gradual process. In a time course cell culture study of fibroblast differentiation from bone marrow mononuclear cells, we observed gradual transition of CD45+ collagen I+ DDR2− bone marrow cells to CD45− collagen I+ DDR2+ fibroblasts in 3 weeks [29]. During this transition, cells at the intermediate stages expressed both CD45 and collagen I. Earlier, Bucala et al. [59] had documented the presence, in circulating blood, of cells intermediate between fibroblasts and hematopoietic cells and named them fibrocytes by drawing analogy from other circulating hematopoietic cells such as erythrocytes and granulocytes. Circulating fibrocytes are CD34+ CD45+ CD11b+ and produce collagen and are thought to be precursors for tissue fibroblasts [59]. In a more recent study of a mouse model of asthma [60], PKH-26-labeled fibrocytes recruited to the airway by repeated exposure to an allergen showed decline of CD34 expression and increase of the intensity of collagen I and αSMA. This in vivo observation is in agreement with our observation of gradual transition of hematopoietic cells to fibroblasts in cell culture [29].

8 Conclusion

This concept of HSC origin of connective tissues contradicts the current dogma that only MSCs give rise to these types of mesenchymal cells. The relationship between HSCs and MSCs regarding tissue-reconstituting abilities needs to be clarified. It is possible that there are dual sources for these mesenchymal cells, i.e., HSCs and MSCs. It is also possible that both HSCs and MSCs are progenies of more primitive stem cells and the apparent tissue reconstitution by HSCs reflects the abilities of the more primitive stem cells that are capable of both hematopoietic and mesenchymal differentiation. For the number of reasons stated in this review, we believe that MSCs are not stem cells and that MSCs are derived from HSCs. This implies hitherto unknown significant tissue-reconstituting potentials of HSCs and possesses major therapeutic implications for diseases and injuries of ligaments, tendons, fat tissues, bones and cartilages.

The new paradigm suggests that transplantation of HSCs, rather than MSCs, is the choice of therapy for such genetic deficiencies of connective tissues as osteogenesis imperfecta, epidermolysis bullosa, Alport syndrome and leptin deficiency. There are indications that BM transplantation is beneficial to patients with severe osteogenesis imperfecta, a collagen I deficiency, [61–63]. Epidermolysis bullosa is caused by collagen VII deficiency and the pathology of a genetic mouse model was ameliorated by transplantation of un-manipulated BM cells from wild-type mice, but not by non-hematopoietic BM cells [64]. Alport syndrome is caused by a deficiency in collagen IV and is characterized by renal insufficiency, deafness and pathological changes in the basement membranes of glomeruli and inner ear. Kidney transplantation is known to be of only limited value. As discussed above, glomerular mesangial cells [14], which produce collagen IV, and inner ear fibrocytes [25] are of HSC origin. Recently, whole BM transplantation was shown to be effective in reversing the renal pathologies in the autosomal Alport mice [65, 66]. The genetic mouse model of leptin deficiency, B6.V-Lepob (ob/ob), develops extreme obesity caused by both hyperphagia and reduced metabolic rate. Since leptin is secreted by the adipocyte, transplantation of normal white adipose tissue that secretes leptin was shown to correct the various defects in this model [67]. We believe that early intervention with HSC transplantation will prevent the development of the severe obesity. In addition to the immediate application of HSC transplantation to the genetic disorders, the new paradigm promises radically new approaches to the development of therapies for many diseases and injuries of connective tissues.

References


