Hematopoietic origin of fibroblasts/myofibroblasts: its pathophysiologic implications

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Tissue fibroblasts/myofibroblasts play a key role in growth factor secretion, matrix deposition, and matrix degradation, and therefore are important in many pathologic processes. Regarding the origin of tissue fibroblasts/myofibroblasts, a number of recent in vivo transplantation studies have suggested the bone marrow as the source of fibroblasts/myofibroblasts in liver, intestine, skin, and lung. Because bone marrow cells are thought to contain 2 types of stem cells (ie, hematopoietic stem cells [HSCs] and mesenchymal stem cells), it is important to determine which type of stem cells is the source of fibroblasts/myofibroblasts. To address this issue, we have carried out a series of studies of tissue reconstitution by single HSCs. By transplanting clones derived from single HSCs expressing transgenic enhanced green fluorescent protein, we found that fibroblasts/myofibroblasts in many organs and tissues are derived from HSCs. This brief note summarizes these findings and discusses clinical and experimental perspectives generated by this newly identified differentiation pathway of HSCs. (Blood. 2006;108:2893-2896)

Introduction

Tissue fibroblasts play a key role in growth factor secretion, matrix deposition, and matrix degradation, and therefore are important in many pathologic processes. For example, at the time of tissue injury, fibroblasts are critical to the inflammatory response and its control. Fibroblasts participate in wound healing by producing extracellular matrix (ECM) proteins and responding to and synthesizing cytokines, chemokines, and other mediators of inflammation (for review, see Eckes et al[1] and Gabbiani[2]). In addition, fibroblasts can be activated to become myofibroblasts, which, because they are armed with myosin and α smooth muscle actin, exert contractile force to reduce the size of the wound. Uncontrolled proliferation and/or activation of these cells results 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. Reviews by Powell et al[3] and Tomasek et al[4] include lists of myofibroblasts and their functions.

Regarding the origin of tissue fibroblasts and myofibroblasts, in vitro evidence for a bone marrow origin was first presented by Friendenstein and associates more than 3 decades ago.[5-8] They identified colonies consisting of fibroblasts by plating bone marrow cells in cultures containing only medium and fetal bovine serum.[5,6] The colonies adhered to culture dishes and consisted of cells exhibiting elongated or polygonal cytoplasm and clear nuclei. The precursors for the colonies were named fibroblast colony-forming units (CFU-Fs) and were detected in a number of tissues, including bone marrow, spleen, thymus, lymph node, and peritoneal and pleural fluids.[7] Subsequently, CFU-Fs were found to possess osteochondrogenic potentials and were thought to be distinct from the hematopoietic lineage.[8] As opposed to the concept of a bone marrow origin of fibroblasts, the model of epithelial-mesenchymal transition (EMT)[9,10] envisioned local generation of fibroblasts from organ epithelium and is based on studies in developmental biology. EMT is thought to be a fundamental mechanism in many embryonic processes wherein epithelial cells break away from the surrounding cells and develop into mesenchymal cells.[11] Recently, however, a number of in vivo transplantation studies have revived the notion of the bone marrow as the source of fibroblasts/myofibroblasts. Using the Y chromosome or green fluorescent protein (GFP) as a marker of donor cells in transplantation studies, investigators have presented evidence that hepatic stellate cells,[12] pericryptal myofibroblasts in the intestine and colon,[13] myofibroblasts in wounded skin,[14] and fibroblasts in pulmonary fibrosis[15] are derived from bone marrow. In functional studies, transplantation of bone marrow cells reduced the magnitude of liver fibrosis that had been induced with carbon tetrachloride.[16] Because bone marrow cells are thought to contain 2 types of stem cells (ie, hematopoietic stem cells [HSCs] and mesenchymal stem cells [MSCs]), it is important to determine which type of stem cells is the source of fibroblasts/myofibroblasts. To address this issue, we have carried out a series of studies of tissue reconstitution by single HSCs. By transplanting clones derived from single HSCs expressing transgenic enhanced GFP, we found that fibroblasts/myofibroblasts in many organs and tissues are derived from HSCs. This brief article is to summarize these findings and to discuss clinical perspectives generated by this newly identified differentiation pathway of HSCs.

Clonal transplantation

To study the full differentiation potentials of HSCs, it is necessary to generate mice exhibiting high-level, multilineage engraftment from

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single HSCs. For this purpose, we devised a method combining single-cell deposition with short-term cell culture.[17,18] Here, Lin^-, $Sca\text{-}1^+$, $c\text{-}kit^+$, $CD34^-$ cells [19] or Lin^-, $Sca\text{-}1^+$, $CD34^-$ side population (SP) cells[20] from GFP mice were individually cultured for 1 week in the presence of steel 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[21] and G-CSF[22] act on cell-cycle–dormant primitive multipotential 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 of incubation significantly raised the efficiency of generating mice with high level multilineage engraftment.[17,18] 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 multilineage engraftment by donor GFP^+ cells were selected for analysis of tissue reconstitution. In order to exclude the possibility that the observed results are artifacts of short-term cell culture, we also carried out transplantation of 100 uncultured Lin^-, $Sca\text{-}1^+$, $c\text{-}kit^+$, $CD34^-$ cells or in each study and made similar observations to those seen in clonally engrafted mice. In most of the studies described, we 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 GFP^+ cells.

The first type of HSC-derived myofibroblasts we detected was glomerular mesangial cells of the kidney.[17] The location in the kidney and morphology of the GFP^+ cells suggested that the cells were mesangial cells. This identification was confirmed by the ability of the GFP^+ cells to contract upon exposure to angiotensin II. Next, we discovered that brain microglial cells and perivascular cells are of HSC origin and demonstrated that induction of stroke by ligation of middle cerebral artery strongly enhanced the recruitment of the GFP^+ microglial cells to the injury site.[18] The morphologic and immunohistochemical properties of the GFP^+ perivascular cells were consistent with the cells being pericytes rather than endothelial cells. We then identified HSC-derived GFP^+ fibroblasts associated with transplantable murine melanoma or Lewis lung carcinoma.[23] The GFP^+ cells were demonstrated to be fibroblasts by their distinct morphology and expression of procollagen 1αI mRNA. In addition, a subpopulation of GFP^+ fibroblastic cells were positive for α smooth muscle actin, indicating they were myofibroblasts. Also prevalent in the specimens were GFP^+ pericyte-like perivascular cells admixed with tumor cells. Similar to our findings in the brain, simultaneous staining for CD31 expression clearly established that the perivascular cells were not endothelial cells. Recently, we found that fibrocytes and other unidentified mesenchymal-type cells in the spiral ligament of the inner ear are of HSC origin.[24] Inner ear fibrocytes are known to play a critical role 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 5 types based on location, morphology, and histochemical properties. GFP^+ cells were seen among all 5 types of fibrocytes.[24] Most recently, we found an HSC origin of the fibroblasts/myofibroblasts in the adult heart valves.[25] Finally, an abstract was presented at the annual meeting of the American Society of Hematology demonstrating an HSC origin of myofibroblasts that are recruited to the injury site following induction of myocardial infarction.[26]

In addition to these in vivo studies demonstrating an HSC origin for tissue fibroblasts/myofibroblasts, we have also succeeded in culture of fibroblasts from GFP^+ bone marrow cells of clonally engrafted mice.[27] GFP^+ 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, generated adherent cells exhibiting the morphology of fibroblasts described 3 decades ago by Friedenstein and associates.[5,6] The GFP^+ cells showed spindle-shaped or pleomorphic cytoplasm and prominent clear nuclei. They also expressed mRNAs for procollagen 1αI, fibronectin, vimentin, and discoidin domain receptor type 2 (DDR2). Time-course flow cytometric analyses of the cultured GFP^+ bone marrow cells revealed gradual expression of collagen I and DDR2 and concomitant loss of CD45 during 3 weeks of incubation. As to the precursors of fibroblasts, both CFU-Fs[5,28] and peripheral blood fibrocytes were derived from the bone marrow of the clonally engrafted mice.[27] This cell-culture study was consistent with the results of in vivo transplantation studies described herein and supported the concept of an HSC origin of fibroblasts/myofibroblasts.

A note of caution is in order regarding the interpretation of these findings. Although our studies demonstrated the differentiation pathway of HSCs to fibroblasts/myofibroblasts, they did not show that all fibroblasts/myofibroblasts are of HSC origin. One possible explanation of this is that the frequencies of GFP^+ fibroblasts/myofibroblasts must be affected by physiologic turnover rates of the fibroblasts/myofibroblasts and that they are likely to vary in each tissues/organs. In the study of solid tumor–associated fibroblasts in a clonally engrafted mouse, we attempted to quantify the frequencies of GFP^+ fibroblasts/myofibroblasts. The percentage of GFP^+ fibroblasts/procollagen $I\alpha l^+$ cells ranged from 0% to 28.6% depending on the images from sequential tissue sections.[23] This observation indicated that a rather large number of fibroblasts/myofibroblasts may be derived from a single HSC. We also observed that injuries such as stroke raise the incidences of HSC-derived fibroblasts/myofibroblasts.[18] While our studies demonstrated an HSC origin for a population of tissue fibroblasts/myofibroblasts that could increase in number cases of insult, it is possible that there are sources other than HSCs for tissue fibroblasts/myofibroblasts.

Several earlier studies have also suggested a close relationship between fibroblasts and the hematopoietic system, in particular the macrophage lineage. It was demonstrated that a population of cells in CFU-F–derived mouse colonies were positive for Mac-1 and F4/80.[29] When $CD34^+$ human peripheral blood or cord blood cells were transplanted into nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice and showed human cell engraftment, the bone marrow was shown to contain $5B5^+$ human fibroblasts and express human proline hydroxylase-alpha mRNA, an enzyme required for collagen synthesis by fibroblasts.[30] In vitro studies have also demonstrated that human peripheral blood cells that express CD14, a surface protein preferentially expressed on monocytes and macrophages, can give rise to fibrocytes when cocultured with T cells.[31] In our laboratory, we examined the correlation between HSC-derived GFP^+ glomerular mesangial cells and B-cell, T-cell, or Mac-1/$Gr\text{-}1^+$ cell populations in clonally engrafted mice. GFP^+ mesangial cells were detected only in the mice expressing Mac-1/$Gr\text{-}1^+$ cells in the blood.[32] In addition, humoral regulation of stromal cells suggests their relation to macrophage lineage. Two groups of investigators observed that macrophage CSF (M-CSF) supports proliferation of fibroblastic stromal cells in culture.[33-36] Although most of these studies are somewhat correlative in nature, they nonetheless suggest closeness between the macrophage lineage and fibroblasts and are consistent with our series of observations in vivo based on clonal HSC transplantation.

Perspectives

The concept that fibroblasts/myofibroblasts are derived from HSCs raises a number of important questions with regard to clinical medicine and basic stem cell biology. First, it appears to conflict with the general belief that, in clinical bone marrow transplantation, bone marrow stromal/mesenchymal cells are of host origin. The overwhelming majority of publications support this concept,[37-44] and fewer studies describe chimerism of marrow stromal cells.[45-49] There may be a number of possible explanations for the apparent discrepancy between our observations and the clinical studies. For example, there may be significant species differences, including radiation sensitivity, between humans and mice. It could also be explained by the differences in the conditioning methods used for transplantation in patients and mice. The apparent disparity among clinical cases may be explained on the basis of different methods for identification of donor stromal cells (ie, cell culture vs polymerase chain reaction [PCR]) and different underlying diseases. For instance, it was proposed that the frequency of mixed chimerism of marrow mesenchymal cells seen in patients with multiple myeloma is due to impairment of bone marrow microenvironment, which is known to be prevalent in this disorder.[50] While the source of these discrepancies is not known, the difficulty in identifying stromal cells of donor origin appears to be unique to bone marrow stromal cells because investigators have documented donor-derived tissue myofibroblasts in the intestine[13] and heart valve[51] of recipients of sex-mismatch bone marrow transplantation. These clinical observations are consistent with our findings of an HSC origin of tissue fibroblasts/myofibroblasts in mice and suggest that fibroblasts/myofibroblasts in many other organs of patients with stem cell transplants may be of donor HSC origin.

Second, the concept of an HSC origin of fibroblasts/myofibroblasts may lead to new avenues of therapy for injury-related disorders. A number of investigators have noted that administration of G-CSF reduces the size of stroke and improves functional outcome in studies of murine models.[52-54] We reported that brain microglial cells and pericyte-like perivascular cells are derived from HSCs, and that they are dramatically increased in number following induction of stroke.[18] G-CSF is known to mobilize HSCs and early hematopoietic precursors to circulation. Taken together, recruitment by G-CSF of precursors for microglial cells and pericytes to the site of injury and their subsequent differentiation to contractile myofibroblasts may be one of the reasons for the reduction in the size of stroke.

Third, the demonstration of HSC-derived fibroblasts/myofibroblasts may clarify some of the controversies surrounding the therapeutic role of HSCs in myocardial infarction. Previously, using a murine model of heart attack, Orlic and his associates reported that transplanted bone marrow cells regenerate infarcted myocardium,[55] and that mobilization of the primitive bone marrow cells by G-CSF to the site of infarct confers therapeutic effects.[56] Subsequently, there has been much discussion on the nature of the HSC-derived cells engrafting the injury site.[57-59] The current consensus appears to be that very few[60] or no cardiomyocytes[59] show donor markers, and that the few cardiomyocytes are the products of cell fusions between donor cells and recipient cardiac cells.[60] Most cells of donor origin in the injury site appear to be fibroblasts/myofibroblasts.[26] Similar to the findings in the murine stroke model, it is possible that the apparent therapeutic effects of G-CSF may be mobilization of fibroblasts/myofibroblasts to the site of myocardial infarction and consequent reduction in the size of scar.[26] Long-term observations of cardiac functions are necessary to confirm the beneficial effects of mobilization of fibroblasts/myofibroblasts by G-CSF. Timely mobilization of fibroblasts/myofibroblasts and facile contraction of scar tissues with cytokines such as G-CSF may hold therapeutic promises to injuries of other organs and tissues.

Finally, the discovery of an HSC origin of fibroblasts/myofibroblasts raises serious questions regarding the current model of stem cell systems in the bone marrow. It has been generally believed that there are 2 types of stem cells in the bone marrow (ie, HSCs and MSCs). HSCs produce blood cells and some cells in the tissues, such as mast cells and osteoclasts. MSCs are responsible for a number of mesenchymal cells, including adipocytes, chondrocytes, and osteocytes, and CFU-Fs are thought to be their precursors.[61-66] While the repertoire of HSC and MSC potential had been thought to be distinct and separate from each other, several studies have begun to question this distinction. Recent studies have shown that a single SP cell and 3000 SP cells can generate osteoblasts in culture and in vivo, respectively.[67] Our observation that CFU-Fs, which had been thought to be progenitors for mesenchymal cells, are also derived from HSCs,[27] further blurs the line between HSCs and MSCs. MSCs are far less clearly defined than HSCs, as pointed out by a recent in-depth review.[68] Indeed, despite significant academic and commercial interest and current ongoing clinical trials, the exact phenotype of MSCs is not known, and most of the studies have been performed in vitro on these "fibroblastic cells." More studies based on transplantation are needed to clarify the relationship between HSCs and MSCs and to delineate their potentials.

In summary, our studies of tissue reconstituting potentials of murine HSCs based on single–stem cell transplantation has uncovered that myofibroblasts in a number of tissues and organs are derived from HSCs. However, we have documented the HSC origin of only several types of myofibroblasts. There are many other types of myofibroblasts (Powell et al[3] and Tomasek et al[4]) that need derivations determined. Our findings may provide a new way of classifying fibroblasts/myofibroblasts and may suggest new clinical interventions for a number of diseases, particularly injury-related disorders.

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