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Adult bone marrow-derived cells: Regenerative potential, plasticity, and tissue commitment

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Abstract

Reconstitution of infarcted myocardium with functional new cardiomyocytes and vessels, a goal that only a few years ago would have been regarded as extravagant, is now actively pursued in numerous laboratories and clinical centers. Several recent studies in animals as well as humans have shown that transplantation of adult bone marrow-derived cells (BMCs) can improve left ventricular function and halt adverse remodeling after myocardial infarction. Differentiation of adult BMCs into cells of cardiac and vascular lineages has been proposed as a mechanism underlying these benefits and, indeed, differentiation of adult BMCs into cells of non-hematopoietic lineages, including cells of brain, skeletal muscle, heart, liver, and other organs, has been documented repeatedly both *in vitro* and *in vivo*. These results are in contrast with conventional definitions and dogma, according to which adult tissue-specific stem cells exhibit only restricted differentiation potential. Thus, these recent studies have sparked intense debate over the ability of adult BMCs to differentiate into non-hematopoietic tissues, and the regeneration of myocardium by differentiation of adult BMCs remains highly controversial. Because of the enormous clinical implications of BMC-mediated cardiac repair, numerous laboratories are currently addressing the feasibility of cardiac regeneration with BMCs and deciphering the mechanism underlying the beneficial effects. The purpose of this review is to critically examine the available evidence regarding the ability of adult BMCs to regenerate non-hematopoietic tissues and their utility in therapeutic cardiac regeneration.

Keywords

myocardial regeneration; stem cell; bone marrow; plasticity; tissue-commitment

Introduction

Extensive preclinical evidence from numerous laboratories supports the notion that adult bone marrow-derived cells (BMCs) can regenerate non-hematopoietic tissues [1–15]. This concept of differentiation ‘plasticity’ of adult stem cells, although supported by strong evidence, is still nascent and has met with enormous skepticism and resistance [16–23]. For many years, embryonic stem cells have been regarded as the only truly pluripotent cells that could differentiate into cells of all lineages in the body. A corollary of this concept is that

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'stem cells' found in adult organs are inherently restricted in their differentiative capacity and irrevocably committed to specific cell types – those of the organ in which they reside. This dogma has been challenged recently.

Indeed, in the past decade, several elegant studies have documented the ability of adult BMCs to differentiate into non-hematopoietic cell types, including brain [1, 5, 11, 24], skeletal muscle [2, 25], liver and epithelium [3, 7, 8], endothelium [26–28], and heart [4, 9, 10, 12]. Anversa's laboratory presented the first evidence [9] that adult lin^{-}/c-kit^{+} BMCs could differentiate into cardiomyocytes, endothelial cell, and vascular smooth muscle cells and regenerate infarcted myocardium. Furthermore, several clinical trials with bone marrow-derived stem/progenitor cells have recently reported encouraging results [29–34]. These findings have enormous implications for patients with ischemic cardiomyopathy and heart failure. The purpose of this article is to critically review this controversial literature and to systematically analyze evidence in favor and against the differentiation plasticity of adult bone marrow-derived cell populations as related to cardiac repair.

Definition of a stem cell

Stem cells are primitive cells capable of self-renewal and differentiation into specific lineages [35]. The zygote and the cells derived from the first two divisions constitute the most primitive cells (totipotent cells) that are able to form the embryo as well as parts of the placenta. The cells of the inner cell mass of the blastocyst can form all of the cell types derived from the three germ layers and are thereby designated as pluripotent cells. The definition of a stem cell beyond this point of differentiation is controversial and has largely been examined in the context of an organ or tissue. It was generally believed that stem cells found in adult organs are already committed to one or a few lineages and are irreversibly restricted in their differentiative capacity. According to this view, the progeny of a stem cell undergoes an irreversible change at a very early stage that makes them permanently committed to a specific downstream pathway of undifferentiated progenitors, committed progenitors, and lineage-committed cells. However, mounting evidence suggests that the initial differentiation into one specific pathway is not so irreversible after all [36]. Although the ability to generate cells of unrelated types decreases in the more committed progenitors, recent findings suggest that the lineage commitment of a stem/progenitor cell is not absolute.

Adult stem cells in the bone marrow

The zygote gives rise to a multicellular individual with numerous organs via a series of events that include differentiation, lineage commitment, cellular proliferation, and programmed apoptosis. However, besides cells with the adult phenotype, many organs also harbor embedded primitive cells that can potentially repair damage due to disease or injury during the life of an individual. These tissue-specific adult stem cells have been described in many organs, including the bone marrow, gastrointestinal tract, skin, lung, liver, and heart. Of these, stem cells in the bone marrow have been studied in the greatest detail. Encased in bones and spread in multiple locations, the marrow is the most protected organ in humans (even more than the brain), and teleologically it would seem appropriate that it would harbor cells that have the potential to regenerate many tissues.

However, the bone marrow is an extremely complex organ with several different types of primitive cells. (i) *Hematopoietic stem cells (HSCs)* are the best characterized population of stem cells in the bone marrow [37]. These cells are negative for lineage markers and express the hematopoietic marker CD45 as well as CD34, CD133, and CD117. HSCs are capable of self-renewal as well as differentiation into all types of mature blood cells. (ii) *Mesenchymal stem cells (MSCs)* are a heterogeneous population of BMCs that grow in cultures as

adherent cells and differentiate into osteoblasts, chondroblasts, and adipocytes [38]. These cells express Stro-1, CD90 (Thy-1), CD106 (vascular cell adhesion molecule-1), and CD13 but not CD45. However, because of a lack of consensus regarding the specific markers expressed by these cells, the true potential for plasticity of MSCs remains poorly defined. (iii) *Endothelial stem/progenitor cells (EPCs)* share a common precursor with the HSCs, the hemangioblast [39, 40]. EPCs express CD34, VEGFR2, Tie-2, and are negative for CD45. Following release from the bone marrow, EPCs circulate in the peripheral blood, home to various adult tissues, and participate in the regeneration of vasculature in adult organs [39, 40]. (iv) *Side population (SP) cells* are identified by their ability to extrude Hoechst dye via the ABCG2 transporter on the membrane [41]. Although SP cells are isolated independently of classic antigenic markers, they are enriched for HSCs. Antigenic characterization suggests that SP cells are a subfraction of the long-term repopulating cells [42]. (v) *Multipotent adult progenitor cells (MAPCs)* are adherent cells that can be cultured indefinitely and possess the potential to differentiate into cells expressing endodermal, mesodermal, and ectodermal antigens. Thus, MAPCs exhibit a highly plastic behavior with a broad differentiation potential [43, 44]. (vi) $CXCR4^+$ *tissue-committed stem cells (TCSCs)* constitute a subpopulation of BMCs that can be isolated by chemoattraction to SDF-1 [45]. These cells express markers for early tissue-committed stem/progenitor cells for skeletal muscle, endothelium, pancreas, liver, and myocardium [12, 45, 46].

BMC-mediated regeneration of non-hematopoietic tissues

In order to evaluate the plausibility of BMCs as a source of tissue regeneration, the available evidence supporting or refuting the ability of BMCs to regenerate adult non-hematopoietic tissues must be examined critically. Although many studies have reported beneficial effects following BMC transplantation, especially with regard to cardiac regeneration, not all studies have provided conclusive evidence that BMCs indeed give rise to non-hematopoietic cells. We will first summarize the evidence in favor of BMC-mediated regeneration of non-hematopoietic tissues followed by the evidence for the contrary.

Evidence in favor of BMC-mediated regeneration

Differentiation into cardiac and vascular lineages

Lin$^-$/c-kit$^\pm$ BMCs: In a landmark study in 2001, Orlic et al. [9] first reported the feasibility of cardiac regeneration with lin^-/c-kit^+ BMCs. Lin^-/c-kit^+ BMCs from male EGFP transgenic mice were injected into the periinfarct region following the induction of myocardial infarction (MI) by permanent coronary ligation in female wild-type mice. Injection of BMCs resulted in regeneration of cardiomyocytes, endothelial cells, and vascular smooth muscle cells resulting in reconstitution of 68% of the infarcted myocardium and formation of new vessels with improvement in left ventricular (LV) function and attenuation of remodeling [9]. Importantly, cardiac regeneration was documented by the expression of EGFP and the presence of Y chromosome in newly formed cells. These regenerated BMC-derived myocytes expressed cardiac-specific transcription factors (Nkx2.5/Csx, MEF2, and GATA-4) and proteins (myosin light chain, troponin T, troponin I, α-myosin heavy chain, atrial natriuretic factor, and desmin). The newly formed cells also expressed connexin 43, indicating the establishment of electrical connection between the regenerated cells and the native cardiomyocytes [9].

MSCs: Several studies have documented the ability of bone marrow-derived MSCs to acquire a cardiomyocytic phenotype in both infarcted [10] and healthy [47] myocardium. In a study by Shake and colleagues [10], porcine MSCs were harvested, labeled with DiI, and injected into the infarct region 2 weeks after MI. Histological examination revealed successful engraftment, evidenced by the presence of a large number of Dil-positive cells in

the myocardium. A significant portion of these cells also expressed α-actinin, troponin T, myosin heavy chain, and phospholamban, indicating differentiation of transplanted MSCs into a cardiac lineage. This was associated with maintenance of wall thickness in the infarct zone in cell-treated animals [10].

In a subsequent study, Toma and colleagues [47] injected *LacZ*-labeled human MSCs into the LV of immunodeficient CB17 SCID/beige adult mice and examined cell engraftment, survival, and cardiomyocytic differentiation. Desmin and cardiac troponin T-positive β-gal$^+$ cells within the myocardium were first identified at 14 days after injection, and all identified MSCs were positive for desmin expression at 60 days, indicating cardiomyogenic differentiation of transplanted MSCs [47].

Elegant work from Fukuda's laboratory has demonstrated that the bone marrow harbors a subset of MSCs with cardiomyogenic potential [48]. Upon treatment with 5-azacytidine, these MSCs express atrial and brain natriuretic peptides, myosin, desmin, and actinin, and differentiate into beating cardiomyocytes. These cells also exhibit cardiomyocyte-like sarcomeres and action potential similar to those of sinus nodal cells and ventricular myocytes. In a subsequent study [48a], the authors generated chimeric mice by reconstituting irradiated wild-type mice with whole bone marrow from EGFP transgenic mice. Following MI and mobilization of BMCs with G-CSF, numerous EGFP$^+$/actinin$^+$ cells were noted in the myocardium. EGFP$^+$/actinin$^+$ cells were also noted in the myocardium when wild-type mice were infarcted and treated with G-CSF following transplantation of purified cardiomyogenic BMCs that expressed EGFP driven by the α-myosin heavy chain promoter. In another elegant study [48b], these investigators isolated cardiomyogenic MSCs by transfection with a recombinant plasmid containing EGFP cDNA driven by the cardiac-specific gene myosin light chain-2v (MLC-2v) promoter. Under these conditions, EGFP was expressed in cells that were programmed to express MLC-2v and could be isolated by flow cytometry. In vitro, these cells expressed cardiomyocyte-specific genes, including α-skeletal actin, β-myosin heavy chain, MLC-2v, and CaV1.2. When injected into normal adult murine myocardium, these cells became integrated into the host myocardium, expressed connexin 43, and survived for at least 3 months. These results provide direct and conclusive evidence that bone marrow-derived MSCs can differentiate into cardiomyocytes.

Multipotent BMCs: The existence of multipotent BMCs that can differentiate into cells of unrelated lineages has been recently described by Losordo and colleagues [15]. These authors reported the identification of clonally derived stem cells from human bone marrow that can maintain multipotency during self-renewal and can differentiate into cells of all 3 germ layers [15]. Following transplantation into infarcted rat myocardium, these DiI-labeled cells expressed markers specific for cardiomyocytes, endothelial cells, and smooth muscle cells and improved LV function and capillary density [15].

Lin$^-$/Sca-1$^+$/CD45−/CXCR4$^+$ myocardial TCSCs: Studies from Ratajczak's laboratory have put forth the hypothesis that the bone marrow serves as a reservoir of primitive cells that are already committed to differentiate into cells of several non-hematopoietic tissues, including heart, liver, brain, muscle, pancreas, and endothelium [45, 46]. Recently, we have demonstrated that under appropriate culture conditions, these TCSCs from adult murine bone marrow can differentiate into cardiomyocytes [12]. Specifically, after 21 days in culture, these cells express cardiac-specific transcription factors (Nkx2.5/Csx, MEF2C, and GATA-4) and structural proteins (cardiac myosin heavy chain and troponin I) and exhibit a cardiomyocytic phenotype [12]. Interestingly, these cells are mobilized into the peripheral blood following acute myocardial infarction in both mice and humans [12, 49]. Although the ability of cardiac TCSCs to repair infarcted myocardium and regenerate vascular structures

remains to be demonstrated, these data indicate that bone marrow-derived TCSCs exhibit the potential to differentiate into non-hematopoietic lineages, including cardiac cells.

EPCs: Although EPCs are present within the bone marrow, the vast majority of work investigating EPC-mediated cardiac regeneration and neovascularization has been performed using EPCs harvested from the peripheral blood. In a seminal study [26], Takahashi and colleagues demonstrated that EPCs are mobilized in response to tissue ischemia and cytokine stimulation and enhance neovascularization in ischemic tissue. In a chimeric mouse model expressing *LacZ* under control of the endothelial-specific Tie-2 promoter in BMCs, bone marrow-derived EPCs enhanced corneal vascularization [26]. Subsequent studies from these investigators have documented myocardial neovascularization by *ex vivo* expanded DiI-labeled human EPCs following intravenous administration after MI in athymic nude rats [27] and following NOGA mapping guided intramyocardial injection of $CD31^+$ nonadherent EPCs in ischemic swine and rat myocardium [28]. In both studies, EPC administration resulted in improvement in LV systolic function [27, 28].

In a rat model of coronary artery ligation, Kocher et al. [50] injected intravenously DiI-labeled $CD34^+$ mononuclear cells from human peripheral blood following G-CSF mobilization. Capillaries of human origin expressing human but not rat CD31 were identified in the infarct zone. This neovascularization resulted in reduced apoptotic cell death and improvement in LV ejection fraction [50]. Interestingly, mobilization of EPCs into the peripheral blood following MI has been reported in humans [51], and differentiation of EPCs into functionally active cardiomyocytes without fusion has been reported in co-culture experiments in vitro [52]. These results suggest that EPCs can differentiate not only into endothelial cells but also cardiomyocytes, and EPC transplantation may induce not only neovascularization but also potentially new myocyte formation. However, since different investigators have used somewhat different methods and markers for the isolation of EPCs, the cell population is not strictly characterized, and this may lead to variable results.

Adherent BMCs: In a rat model of myocardial cryoinjury, Tomita and colleagues transplanted autologous adherent BMCs for cellular cardiomyoplasty [4]. Eight weeks later, transplanted BMCs expressed muscle-specific proteins and improved systolic and diastolic function in rats that received BMCs pretreated with 5-azacytidine [4]. However, the specific BMC subset responsible for cardiomyogenic differentiation could not be identified from this study because of the use of an unfractionated BMC population.

Differentiation into neural lineage—Several studies have shown the ability of BMCs to trans-differentiate into cells of the central nervous system, including microglia, astrocytes, and oligodendrocytes [1, 5, 24]. Eglitis and Mezey [1] transplanted adult female mice with BMCs genetically marked with a retroviral tag or marrow from male donors. *In situ* hybridization histochemistry and immunohistochemical staining for lineage-specific markers identified BMCs in the brains of the recipients. Some of the BMCs expressed markers for microglia and astroglia [1]. Subsequently, Brazelton et al. [24] and Mezey et al. [5] simultaneously reported the detection of numerous BMC-derived neuronal cells following transplantation of irradiated wild-type mice with marrow from EGFP transgenic mice. The presence of Y chromosome in cells positive for the neuronal markers provided compelling evidence in favor of neuronal differentiation of donor-derived BMCs [5].

In a study by Zhao et al. [11], following injection into the periinfarct areas in the cortex of infarcted rats, transplanted human MSCs expressed markers for astrocytes, oligodendroglia, and neurons and this was associated with amelioration of neurological deficits. However, morphologically, these regenerated cells maintained a spherical phenotype without apparent

integration within the host nervous system [11]. Thus, despite repeated documentation of differentiation of BMCs into neuronal phenotypes, a clear association of such differentiation with functional improvement remains to be established.

Differentiation into skeletal muscle lineage—Ferrari et al. [2] first described transdifferentiation of BMCs into skeletal muscle. Unfractionated BMCs from C57M/*LacZ* transgenic mice were injected into the chemically injured tibialis anterior muscle of immunodeficient mice. Two to five weeks later, 4 of 6 mice that received unfractionated BMCs exhibited β-gal$^+$ nuclei within the damaged muscle [2]. After bone marrow transplantation, intravenously injected BMCs also homed into the damaged muscle and differentiated into a skeletal muscle phenotype [2]. Subsequent studies with mouse models of Duchenne muscular dystrophy demonstrated that *mdx* transgenic mice transplanted with wild-type bone marrow contained donor-derived cells that were positive for muscle-specific markers (myogenin and Myf-5) and expressed dystrophin [25]. Similarly, when irradiated mice were transplanted with EGFP$^+$ BMCs, BMC-derived satellite cells and multinucleated muscle fibers could be identified in recipient mice. Although these studies indicate that BMCs can differentiate into a skeletal muscle phenotype, the specific type of BMC that exhibit this plastic behavior remains to be identified.

Differentiation into hepatic and epithelial lineages—In sex-mismatched and cross-strain transplantation experiments, Petersen and colleagues [3] first documented the ability of BMCs to differentiate into hepatic oval cells. Following sex-mismatched whole bone marrow transplantation in irradiated female mice, Theise and colleagues [7] were able to identify Y chromosome positive donor-derived hepatocytes in recipients. Simultaneous fluorescent *in situ* hybridization for Y chromosome and albumin mRNA identified these cells to be mature hepatocytes [7]. In patients, examination of autopsy and biopsy specimens from female recipients of bone marrow from male donors and male recipients of orthotopic liver transplant from female donors revealed a relatively high percentage of BMC-derived hepatocytes and cholangiocytes [8]. In a subsequent study, Lagasse et al. [6] showed that intravenous injection of a small number of c-kit^{high}/Thy^{low}/lin$^-$/Sca-1$^+$ HSCs from ROSA26 mice could regenerate functional liver tissue and rescue the phenotype in mice with fatal hereditary tyrosinemia type 1. Several other recent studies have demonstrated differentiation of donor-derived BMCs into hepatic and dermal tissue in humans [53] and in sheep [14]. Other studies have reported the generation of epithelial cells from donor-derived BMCs throughout the gastrointestinal tract in humans [54].

Evidence against BMC-mediated regeneration

As is apparent from the above discussion, there is over-whelming evidence in favor of the ability of BMCs to give rise to non-hematopoietic cells and tissues [1–15]. Despite this, several prominent laboratories have been unable to detect significant regeneration of other cells/tissues with BMCs [16, 21, 22]. In some instances, the extent of BMC-dependent regeneration has been extremely small, and such a low rate of differentiation would not account for the observed functional benefits. Moreover, it has been postulated that fusion between native cells and BMCs represents the major mechanism underlying the apparent ‘differentiation’ or ‘regeneration’ of non-hematopoietic tissues by BMCs [17–20]. Collectively, these studies question the ability of BMCs to differentiate into cells of non-hematopoietic lineages.

To evaluate the non-hematopoietic differentiative potential of c-kit$^+$/Thy1.1^{lo}/lin−/Sca-1$^+$ HSCs, Wagers et al. [16] reconstituted irradiated wild-type mice with a single EGFP$^+$ HSC. Despite successful hematopoietic reconstitution, histological examination failed to detect any appreciable contribution of donor-derived EGFP$^+$ cells to non-hematopoietic tissues of

the recipient mice. Engrafted $EGFP^+$ cells retained the hematopoietic marker CD45 and did not express any antigenic marker specific for skeletal muscle, lung, intestine, or brain [16]. However, a few donor-derived hepatocytes were noted in the liver and a single donor-derived Purkinje cell was found in the brain [16]. The authors concluded that adult HSCs do not exhibit developmental plasticity and are incapable of regenerating non-hematopoietic tissues. A major problem with this study is that the progeny of a single HSC may not be able to give rise to non-hematopoietic tissues in the recipient; transdifferentiation may require the concurrent combinatorial actions of several different types of BMCs normally present in the bone marrow, which may not all be reconstituted from a single HSC.

In a subsequent study [21], investigators from the same laboratory examined the cardiomyogenic potential of different subsets of c-kit enriched BMCs in a mouse model of myocardial infarction. C-kit^+, lin^-/c-kit^+, and c-kit^+/Thy1.1^{lo}/lin^-/Sca-1^+ cells from EGFP transgenic mice were injected directly into the ischemic myocardium. At 10 days after MI, a large number of BMCs were detected in the myocardium but this number decreased drastically at 30 days after MI [21]. Despite this, there was significant improvement in LV function and chamber dilatation in cell-treated mice compared with saline-treated controls at 6 weeks after MI. When a parabiotic model was surgically created immediately after MI in a wild-type mouse, establishment of chimerism could be demonstrated and $EGFP^+$ cells derived from the conjoined EGFP transgenic mouse were noted in the myocardium [21]. However, these parabiotic partner-derived cells did not express markers for cardiac, endothelial, or smooth muscle cells [21]. A major problem with this study is the fact that the reported mortality at 30 days following permanent coronary occlusion in mice was only 9–14% in all groups, which is extremely unlikely even under the best possible experimental conditions. In addition, the success rate for intramyocardial injection of cells was not specified; this is a technically challenging technique that, even in the best hands, is only partly successful. Finally, the sample sizes used in some experiments were very small (2–4 mice), which is clearly not sufficient for statistically sound conclusions.

In another study by Murry et al. [22], lin^-/c-kit^+ BMCs from transgenic mice expressing a nuclear-localized β-galactosidase reporter gene driven by the α-myosin heavy chain promoter were transplanted into the periinfarct region in wild-type mice. No transdifferentiation of genetically labeled BMCs into cardiomyocytes was noted. *In vitro* co-culture experiments also failed to detect any transdifferentiation of BMCs into cardiac cells [22]. The authors concluded that HSCs do not transdifferentiate into cardiomyocytes. Again, as in the previous study, the success of intramyocardial injection of cells was not reported in this study.

The reason for the discrepancy in the results from different laboratories [9, 21, 22] remains unknown. The aforementioned methodological problems with the studies of Balsam et al. [21] and Murry et al. [22] could be responsible, at least in part, for these differences. It should be noted that even with a sophisticated genetic marker system, the quantitation of differentiation remains highly dependent on the techniques used [55]. Only the most meticulous assessment with the most reliable tools yields reproducible and reliable data [55]. In addition, an alternative theory has been advanced by some investigators who claim that the ‘transdifferentiation’ phenomenon is a result of cell fusion [18–20]. Indeed, fusion of donor BMCs with hepatocytes, Purkinje cells, and cardiomyocytes of the recipient has been documented [18, 19]. Although cell fusion occurs during normal embryonic and fetal development, it is a rare event in adult animals and humans except in a few specialized cell types [56, 57]. Subsequent studies using BMCs [58] as well as other adult primitive cell types [59, 60] have excluded any major contribution of cell fusion to cardiac regeneration. The conjecture that cell fusion could account for the apparent cardiac regeneration observed is far-fetched for several reasons. First, newlyformed myocytes are small [9, 61], which is

not consistent with the concept that BMCs fuse with large, preexisting myocytes. Second, the number of myocytes left in the infarct following a permanent coronary occlusion is very low, and not sufficient to account for the large number of newly formed myocytes observed. Third, and perhaps most important, the speculation that fusion of a BMC with a pre-existing myocyte would improve cardiac function is not supported by any available evidence. Given the overwhelming evidence in favor of a beneficial effect of BMC transplantation on LV function and postinfarct remodeling in patients [29, 30, 32, 33, 62], these negative results [21, 22] must be interpreted with extreme caution in order not to ignore the potentially vast therapeutic implications of this strategy.

Plasticity vs. tissue-commitment

For many years, the HSCs have been known to have proliferative as well as differentiative capacities [37]. This ability to self-renew and give rise to several different types of hematopoietic cells was previously regarded as plastic behavior. However, based on the recent demonstration that HSCs can cross lineage boundaries and give rise to cells of non-hematopoietic lineages, the definition of stem cell plasticity has been modified. According to this new view, a stem cell is considered ‘plastic’ only if it can switch lineages and acquire the phenotype of a primitive cell or an adult cell from a different lineage. This scenario also includes events where a stem cell crosses embryologic lineage boundaries and switches phenotype between endodermal, mesodermal, and ectodermal lineages. This plastic behavior of a stem or adult cell may be a manifestation of dedifferentiation, transdifferentiation, transdetermination, or cellular fusion [63, 64]. Although the underlying mechanisms remain to be elucidated, these events may be influenced by local environmental factors that include interactions with the stroma/matrix and growth factors/cytokines, genetic programming, or stochastic mechanisms. The contribution of these mechanisms to cellular plasticity *in vivo* is difficult, if not impossible, to determine precisely.

Apart from the aforementioned mechanisms by which bone marrow-derived primitive cells can potentially regenerate cardiac and other non-hematopoietic tissues, recent compelling evidence supports a new mechanism, namely, the concept of TCSCs [12, 45, 46, 65–67]. Previous studies have indicated that the bone marrow harbors a subset of primitive cells that exhibit the potential to differentiate into several different unrelated lineages [43, 44, 68–70]. Although differentiation of BMCs into different unrelated tissues could be triggered by the microenvironment following homing, studies from Ratajczak’s laboratory and our laboratory have demonstrated that a subset of small $CXCR4^{+}/Sca\text{-}1^{+}/lin^{-}/CD45^{-}$ BMCs express mRNA transcripts and proteins specific for skeletal muscle, neural, endothelial, hepatic, pancreatic, and cardiac lineages [12, 45, 46]. It appears that in anticipation of tissue damage and the need for organ repair, nature has created a repository of primitive cells in the bone marrow that are already destined to regenerate diverse tissues in adults. These cells can be mobilized into the peripheral blood in response to tissue damage [46, 49] and home to the injured organ with subsequent differentiation into adult tissue-specific cells. Tissue-commitment of BMCs to regenerate diverse non-hematopoietic tissues would explain the observation of non-hematopoietic tissue reconstitution by BMCs, without invoking transdifferentiation. The notion that tissue-committed cells exist in the bone marrow may potentially reconcile the apparent conflict between those who support ‘plasticity’ of bone marrow-derived stem cells, those who support cell fusion, and those who deny both of these phenomena.

Clinical implications of BMC therapies

Reconstitution of diseased or aging human organs with new cells has been the holy grail of regenerative research for many years. The ability to reconstitute cardiac tissue is of paramount importance. An estimated 5 million people in the United States suffer from

congestive heart failure, and approximately 450,000 new patients are diagnosed every year [71]. The vast majority of these cases are the result of myocardial infarction, which leads to ischemic cardiomyopathy with progressive adverse LV remodeling [72]. Although interventional strategies to achieve coronary reperfusion and conventional pharmacologic therapies for heart failure can alleviate symptoms and reduce mortality, they fail to reconstitute lost myocardium. Several recent studies have shown the ability of adult cardiac stem cells (CSCs) to repair infarcted myocardium and restore function in animals [59, 60]. Although CSCs are ideally suited for cardiac regeneration, the use of BMCs offers an alternative approach if CSCs cannot be procured in a timely fashion or are otherwise defective.

Because of the overwhelming clinical need to improve LV function and survival in millions of patients with myocardial infarction and ischemic cardiomyopathy, the knowledge gleaned from animal studies has been rapidly applied to the clinical setting. Thus far, small and mostly non-randomized clinical trials have used bone marrow-derived mononuclear cells [29, 32, 34], EPCs [30, 33, 62], and cytokine-induced mobilization of BMCs [73–75]. These studies have demonstrated the feasibility of harvesting, purifying, and transplanting BMCs as well as of mobilizing BMCs with cytokines in patients with acute MI and ischemic cardiomyopathy. Notwithstanding the fact that it is very arduous to detect differentiation of transplanted cells in humans, virtually all clinical investigations reported to date have demonstrated benefits in terms of improvement in LV function and amelioration of adverse LV remodeling with the use of BMCs, suggesting that even without identification of the precise cell type and the underlying mechanism of regeneration – trandifferentiation vs. tissue-commitment vs. fusion – BMC therapy can alleviate myocardial damage in humans. Even more importantly, the experience accumulated thus far in almost 200 patients that have received BMCs following acute MI has shown no significant adverse effects [76, 77], indicating that autologous BMC transplantation is safe. These data provide a compelling rationale for proceeding with phase II and III randomized, double-blinded clinical trials to conclusively assess the efficacy of BMC transplantation for improving ventricular function and mitigating LV remodeling after MI.

Despite their efforts, the opponents of BMC therapy for cardiac regeneration will not be able to stop scientific progress. Nobody can. The encouraging results of the initial phase I-II clinical trials of BMC transplantation [76, 77] have ignited an explosion of research in patients with both acute MI and chronic ischemic cardiomyopathy. Halting, as some have proposed [23], the investigation of a promising and safe therapy, which has enormous potential, simply because some mouse studies [21, 22] have been negative or because we do not yet understand the exact mechanism would be absurd and, indeed, unethical. Surely it would be nice to know how BMC therapy works. But should we stop clinical investigation until we resolve this issue (which will require, most likely, many years)? How many therapies do we use whose mechanism of action is fully understood? For example, do we know exactly how statins, ACE inhibitors, and beta-blockers, just to cite a few major examples, reduce cardiovascular events? Would anyone suggest we ought to stop using these drugs (which, at the moment, appear to have more side effects than BMC therapy) until we elucidate their mechanism of action? And who will tell a patient dying of heart failure that he/she will be denied access to a promising and safe therapy because we have not yet resolved the mechanism?

In summary, the initial clinical results [29, 30, 32–34, 62, 73–75] are extremely promising, and future larger studies may lead to the formulation of effective therapeutic strategies so that BMC-mediated cardiac regeneration can be implemented on a routine basis.

Conclusions

Repair of the infarcted heart remains one of the major goals of current regenerative research. Over the last decade, numerous studies have documented the ability of adult BMCs to differentiate into non-hematopoietic lineages, including neural, skeletal muscle, hepatic, and cardiac and vascular lineages. With regard to cardiac repair, beyond mere histological findings, improvement in LV function and structure following BMC transplantation has been noted in both animal and human studies. While controversy persists regarding the ideal cell type (bone marrow mononuclear cells, $lin^{-}/c\text{-}kit^{+}$ BMCs, MSCs, EPCs, MAPCs, or TCSCs) and the underlying mechanism of cellular regeneration (transdifferentiation and plasticity vs. tissue-commitment vs. fusion vs. paracrine effects), the overwhelming body of evidence favoring a beneficial role of BMCs in cardiac disease cannot and should not be ignored. Critical examination of the available evidence from basic and clinical studies strongly supports the notion that BMC-mediated cardiac regeneration is feasible and safe. Randomized, doubleblinded studies will be necessary to establish the therapeutic efficacy and the safety of BMC administration for cardiac repair. Furthermore, future studies comparing the efficacy of different subsets of BMCs will be necessary to formulate an effective therapeutic strategy for cardiac repair in humans. The therapeutic nihilism of those who oppose clinical studies of BMC therapy should not be allowed to stop the progress of this promising field.

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