Editorial

Bone marrow cells for cardiac regeneration: the quest for the protagonist continues

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See also article by Hattan et al. [11] (pages 334–344) in this issue.

1. Introduction

The past few years have witnessed an explosion of research on methods to achieve myocardial repair via cellular transplantation or mobilization of endogenous cells [1]. Several different cell types, including skeletal myoblasts [2], c-kit+/Sca-1+/lin− bone marrow (BM) cells [3], BM mesenchymal stem cells (MSCs) [4], BM ‘side-population’ (SP) cells [5], endothelial progenitor cells (EPCs) [6], and cardiac stem cells (CSCs) [7], have been utilized in an effort to achieve cardiac reconstitution and restoration of function following myocardial infarction (MI). The evidence accumulated over the past few years indicates that left ventricular (LV) function and adverse LV remodeling can be ameliorated via cell-based therapies, and the preliminary results from several small-scale, mostly non-randomized, clinical trials have been encouraging [8–10]. However, the mechanism(s) underlying the observed functional improvement remains unclear and the debate regarding the advantages of one candidate cell versus another continues unabated.

2. The ideal cell for therapeutic use

So, what characteristics should a cell have in order to be selected for therapeutic use in humans? The answer is simple: it has to differentiate into cardiomyocytes that are connected both mechanically and electrically with native myocytes (contracting in synchrony with adjacent cells) and be able to form vascular structures in order to get blood supply in the scar. Thus far, very few cell types have been shown to do all of these things.

In this issue of the Journal, Hattan, Kawaguchi, and colleagues report the isolation and purification of a ‘cardiomyogenic’ subpopulation from BM MSCs [11]. MSCs were treated with 5-azacytidine followed by transfection with an EGFP cDNA driven by the myosin light chain (MLC)-2v promoter. EGFP-positive cells were isolated by flow cytometry. These cells expressed cardiomyocyte-specific genes in culture, incorporated BrdU, exhibited sarcomeric organization, and demonstrated spontaneous beating after 3 weeks. Electrophysiologic studies revealed a cardiomyocyte-like action potential. Following transplantation into non-injured mouse hearts, EGFP-expressing cardiomyocytes could be detected within the myocardium. The findings are important because they raise the possibility of using large-scale isolation of BM-derived cells destined to differentiate into cardiomyocytes for therapeutic use in myocardial repair. However, before this type of preselection can be applied to humans, several issues need to be resolved. First, although the expression of MLC-2v and the concomitant expression of a marker identify a cell as ‘cardiomyogenic’, these cells have not been characterized with respect to antigen expression and lineage commitment. Previous studies of BM cells have yielded conflicting results. For example, evidence has been provided that c-kit+/Sca-1+/lin− cells differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells following intramyocardial injection [3] and that BM-derived SP cells also transdifferentiate into cardiomyocytes and endothelial cells.

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[5]. Others, however, have reported that c-kit+/Sca-1+/Thy1.1−/lin− long-term repopulating hematopoietic stem cells failed to differentiate into anything but adult hematopoietic lineages [12]. So, to which subpopulation do these cardiomyogenic cells belong? A thorough phenotypic characterization of these cells may help to resolve these issues. Second, it is doubtful that EGFP labeling can be used for selecting cells for human therapeutic use, since overexpression of EGFP or other cytoplasmic proteins can potentially cause adverse alterations in the cellular milieu [13]. An ideal marker would be one that is expressed on the cell surface and helps sorting of positive cells by flow cytometry. Third, the ultimate proof-of-principle in cardiac ‘regeneration’ lies in the demonstration that cardiac function improves following transplantation into the damaged myocardium. Although these cells express connexin 43 in culture and exhibit a cardiomyocyte-like action potential, it remains to be proven whether following transplantation into the infarcted myocardium they will also survive and improve LV function. Finally, an alternative to the hypomethylating agent 5-azacytidine should be explored to address any potential concern regarding tumorigenesis [14,15].

3. Regeneration of the vasculature

Another unanswered question revolves around the issue of blood supply to the dead or regenerated myocardium. The current literature does not specifically address the importance of cardiomyocyte regeneration vis-à-vis vascular regeneration. Peripheral blood-derived EPCs have been successfully employed to improve myocardial function [6,16], and cardiomyocyte regeneration with scar repair has been reported to occur without vascular differentiation [4]. So, do we necessarily need to select only those cells that generate cardiomyocytes but not vessels? The answer is presently unclear. However, an alternative has been offered by Anversa’s group that reported the existence of resident CSCs that differentiate not only into cardiomyocytes but also into endothelial cells and smooth muscle cells [7]. Because of their ability to differentiate into several different cell types, CSCs offer a more balanced reparative approach that would not only restore ventricular function, but also secure blood supply for continued growth and sustained function amidst a bloodless scar. As the natural progenitor of cardiac cells, the CSC would appear best suited for the complex task of reconstituting tissue that is lost after a myocardial infarction [7].

4. The existence of ‘tissue-committed stem cells’ (TCSCs) in the BM

The selection process used by Hattan, Kawaguchi, and colleagues was based on the ability of BM MSCs to express MLC-2v [11]. These results raise a very fundamental question: is it possible that these cells were already expressing MLC-2v even before the harvest and thereby were “committed” to differentiate into cardiomyocytes anyway? Does the adult BM harbor cells that are ready to repair other tissues in the body when necessary? Ratajczak et al. [17] have recently demonstrated the existence of cells in the adult BM that express markers for various adult tissues, including brain, skeletal muscle, and endothelial cells. These TCSCs express CXCR4 and can be isolated by chemotraction to stromal-derived factor-1 [18]. Intriguingly, we [19] have recently identified a subpopulation of BM-derived CXCR4+/Sca-1+/lin−/CD45− cells that express not only GATA-4, Nkx2.5/Csx, and MEF2C but also cardiac-specific myosin heavy chain and troponin I in culture, and thus appear to be cardiac TCSCs. These cardiac TCSCs are present in both mice and humans [19]. Recently, cardiac, endothelial, and skeletal muscle TCSCs have been shown to be mobilized following MI in humans [20]. The ability to select cardiomyogenic cells on the basis of promoter activation, as shown by Hattan, Kawaguchi, et al., supports the concept that within the adherent cell population in the BM reside subpopulations of TCSCs that can be identified by their ability to express tissue-specific proteins. These TCSCs for multiple tissues can be potentially selected or isolated from the BM, expanded in culture, and utilized for organ repair.

5. Future directions

Regenerative medicine is still in its infancy and many hurdles need to be overcome before effective repair of the heart becomes a reality. The study by Hattan, Kawaguchi, and colleagues [11] is important because it provides solid evidence that the adult BM contains cells that can be selected on the basis of cardiac-specific gene expression and that these cells not only survive following transplantation but also interdigitate with the native cardiomyocytes. Further studies will be necessary to investigate the nature of these cells vis-à-vis other BM cells, such as the recently described cardiac TCSCs [19], to determine whether regeneration of cardiomyocytes alone is sufficient to improve LV function following MI, and to establish whether therapy with these cells offers any tangible advantage over CSCs with respect to myocardial regeneration.

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