Editorial

Questioning the relevance of circulating cardiac progenitor cells in cardiac regeneration

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See article by Ausoni et al. [10] (pages 394–404) in this issue.

1. Introduction

Regeneration of injured myocardium would offer relief to millions of patients with left ventricular dysfunction. Cell implantation and induction of stem cell-mediated myocardial self-repair represent potential means to achieve myocardial regeneration[1,2]. The latter requires homing of cardiogenic cells to diseased myocardium or activation of resident cardiogenic stem cells. Both mechanisms have been described in the literature and would be preferable to exogenous cell engraftment with its drawbacks of invasive cell harvesting, cell propagation, and invasive cell deposition in the heart [3–6]. However, recent studies questioned the principal capacity of circulating adult stem cells to give rise to cardiac myocytes and support myocardial regeneration [7–9]. In the current issue of Cardiovascular Research, Ausoni et al. provide further evidence against the role of circulating stem cells in cardiac regeneration [10].

2. Finding stem cells in the heart

Assessment of stem cell-mediated cardiac regeneration requires unambiguous identification of these cells and their progeny in the heart. Several markers, including c-kit, sca-1, and isl-1, have been described to denote cardiac precursor cells, but are lost once progenitors differentiate into somatic cells, making cell fate mapping difficult [3–5]. Stable, preferably genetic, marking of stem cells and their progeny is thus a requirement if the fate of an individual cell is to be followed from the multipotent progenitor state to the differentiated somatic cell. Quiani et al. were the first to utilize sex-mismatched heart transplants (donor: female, recipient: male) and Y-chromosome-specific fluorescence in situ hybridisation to detect male cells in female transplants, thereby providing evidence for the capacity of cells of extracardiac origin to home to the heart [11]. Several groups principally reproduced these pivotal findings, but came up with quantitatively different results (chimeric cardiac myocytes: 18% [Quiani et al.] vs. 0.04% [LaFlamme et al.]) [12–14]. In an earlier study, Jackson et al. engrafted β-galactosidase-expressing hematopoietic bone marrow stem cells into lethally irradiated wild-type recipient mice and assessed whether these cells home to the heart and generate cardiac myocytes [15]. In this study 0.02% of the cardiac myocytes were β-galactosidase-positive in infarcted hearts, suggesting that bone marrow-derived cells indeed contribute to the generation of cardiac myocytes. Yet, identification of endogenous (Y-chromosome) or extrinsic (β-galactosidase) markers in cardiac myocytes may not be conclusive evidence for transdifferentiation, as they could also result from fusion of progenitor cells with preexisting cardiac myocytes [16]. To distinguish these events, Nygren et al. reconstituted lethally irradiated Rosa26 mice (ubiquitous expression of β-galactosidase) with bone marrow from green fluorescent protein (GFP)-expressing transgenic animals [9]. In this model, cell fusion leads to simultaneous expression of β-galactosidase and GFP, and transdifferentiation would result in β-galactosidase-negative, GFP-positive cardiac myocytes. Notably, the authors could not identify a single trans-
differentiation event, but identified cell fusion with endogenous cardiac myocytes at a rate of 0.0065%, a value roughly comparable to the data from Jackson et al. [15].

In the current issue of *Cardiovascular Research*, Ausoni et al. employed another elegant model to test the role of circulating stem cells in myocardial regeneration, namely heterotopic transplantation of wild-type rat hearts into ubiquitously GFP-expressing recipients [10]. Appearance of GFP-positive cells in these hearts indicates host-cell homing. A key finding of the study was that only 0.005–0.008% of the cardiac myocytes were of recipient origin (GFP-positive). In addition, GFP-positive endothelial cells, but not smooth muscle cells, were identified in the transplanted hearts. Granulocyte colony-stimulating factor (G-CSF) treatment did not enhance the number of GFP-positive cells in the transplants. Detailed studies by confocal microscopy revealed that all of the “cardiac regeneration events” resulted from cell fusion rather than de novo formation of cardiac myocytes. Interestingly, all GFP-positive cardiac myocytes contained more than 2 nuclei (85% 4–6 nuclei, 15% 3 nuclei), a finding that was only observed rarely in native myocardium (9% >2 nuclei). Taken together, Ausoni et al. provide strong data arguing against the role of circulating stem cells in cardiac regeneration.

3. Strengths and limitations of the study

Principally, Ausoni et al.’s study recapitulates the earlier findings of Jackson et al. [15] and Nygren et al. [9]. However, there is additional merit in the present study. First, the experiments were conducted in another species and yielded similar results. Second, depletion of the bone marrow by irradiation, as employed by the previous studies, may not always be complete or may alternatively lead to destruction of stem cell niches that may not be repopulated properly after bone marrow reconstitution. Both events would lead to an underestimation of the regeneration capacity of bone marrow derived progenitor cells. In contrast, the present study used genetically labeled, but otherwise healthy and naïve rats. Moreover, heterotopic transplantation, as employed by Ausoni et al., is unlikely to affect the general regenerative capacity of the recipient’s bone marrow cells. Yet, additional limitations must be considered:

1. Tissue damage under heterotopic transplantation differs from myocardial damage after myocardial infarction. In fact, unloading of heterotopic hearts leads to atrophy with structural deterioration of the myocardium. In contrast, infarcted orthotopic hearts respond initially with compensatory hypertrophy. A variety of cytokines/chemokines are released locally from infarcted hearts and may attract circulating stem cells under pathophysiological conditions. Heterotopic hearts may not liberate a similar growth factor cocktail, leading to reduced homing of cardiac progenitors. Differences in the local paracrine milieu may also explain why G-CSF had no effect on GFP-positive cell number in the hearts despite a massively increased number of circulating progenitor cells. Finally, the necessity to use cyclosporine in this model of cardiac allotransplantation may be a potential caveat.

2. Transgenic modification of cells might alter their physiologic function. Notably, all GFP-positive cardiac myocytes showed more than 2 nuclei. Although binucleation is common in adult rat cardiac myocytes, the presence of more than two nuclei is only rarely observed. In mice, cell fusion between native cardiac myocytes and Sca-1-positive progenitor cells did apparently not lead to comparable polynucleation [5]. The observation of multinucleation after cell fusion in the study by Ausoni et al., observed in all GFP-positive cardiac myocytes, may indicate genomic alterations in GFP-transgenic cells that may inhibit nuclear fusion and could also influence their general transdifferentiation capacity.

4. Conclusion

Recent data have challenged the dogma that the heart is incapable of self-regeneration. Two main endogenous sources for cardiogenic adult stem cells are currently discussed. These are (1) circulating bone marrow-derived stem cells and (2) resident cardiac progenitors [3–6]. In the current issue of *Cardiovascular Research*, Ausoni et al. provide strong data arguing against a significant role of circulating stem cells in myocardial regeneration, in agreement with several other recent reports [7–9]. The strength of the study is the use of GFP-expressing recipient rats in a heterotopic heart transplantation model with unimpaired bone marrow function. On the other hand, the main criticism also relates to the utilized model of heterotopic transplantation, which may not simulate the pathophysiologically relevant situation of myocardial infarction or heart failure. Regardless of this critique, this study provides important data supporting recent findings that question the role of circulating stem cells in cardiac regeneration and will certainly add to the lively discussion concerning the general role of stem cells in cardiac regeneration.

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References


