Circulating Around the Tissue
Hematopoietic Cell–Based Fusion Versus Transdifferentiation

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The battle of life is, in the most cases, fought uphill...

Samuel Smiles

Clinical application of bone marrow–derived cell populations remains one of the most popular sources for the treatment of ischemic heart disease around the world. Studies initially reported by Orlic et al,1,2 described the exceptional ability of hematopoietic stem cells (HSCs) to transdifferentiate into cardiomyocytes and microvessels in the absence of cell fusion after cytokine stimulation, myocardial infarction (MI), and postadoptive transfer into the damaged heart. The cellular therapeutic research field has displayed hesitancies in establishing the regenerative capacity of hematopoietic cells, confounded by the streamlined use of bone marrow–derived cell types in clinical trials. In addition, there remain questions as to the efficacy of bone marrow–derived stem cells after suggested discrepancies during meta-analysis of clinical data end points obtained in the past decade.3 Nonetheless, as many studies that conclusively support bone marrow cell (BMC) transdifferentiation, there are an equal number of reports refuting significant de novo formation of cardiomyocytes from HSCs.4–7 In 2004, both Murry et al8 and Balsam et al9 did not observe transdifferentiation of CD45+/Lineage−/c-kit+ to the level that was reported by Orlic et al1,2 after direct injection in the infarcted myocardium. Balsam et al8 suggested an additional experimental model for analyzing the commitment of endogenous circulating stem cells after infarction injury using mouse parabiosis, the process of surgically joining 2 mice to promote shared blood circulation. A new study in this issue of Circulation Research suggests the initial data from the study by Balsam et al9 underestimate the contribution of circulating cells to cardiomyocytes after injury because of the timing of surgical parabiosis relative to MI.

In the recent study by Wu et al9 the researchers challenge a long-standing parabiosis model and re-evaluate the contribution of circulating BMCs to give rise to cardiomyogenic structures in vivo. The main issue with the original article by Balsam et al9 was in the timing of parabiosis and myocardial damage, which raises disparate results in reference to BMCs acquiring myocyte fate. In the study by Balsam et al,8 it was found that artery ligation was induced in a wild-type mouse followed by an immediate surgical parabiosis to the ubiquitous green fluorescent protein (GFP) transgenic mouse. Wu et al9 claim that without established cross-circulation for ≥7 days before MI, the recruitment of BMCs would be insufficient to observe GFP cells in the heart, let alone transdifferentiation or fusion events within the myocardium. Changes in the parabiosis experiment, such as establishing shared circulation for ≤1 month, revealed that circulating cells can give rise to an observable amount of vascular endothelial and smooth muscle cells in vivo.9 Interestingly, a majority of GFP cells (>90%) maintained the expression of bone marrow–derived markers, including the pan-hematopoietic marker CD45, 2 weeks after damage, consistent with previous studies.6,8–10

To account for the small but significant number of GFP cells in the heart that acquire cardiac fate by transdifferentiation or cell fusion, 2 separate models were used. The first experiment used an α-myosin heavy chain promoter–driven Mer-Cre-Mer mouse as the recipient mouse, which was surgically attached to a donor GFP transgenic mouse.9 Although significant fusion of GFP cells with existing cardiomyocytes (GFP+/Cre−) was observed, 0.17% of GFP cardiomyocytes arose from transdifferentiation events (GFP+/Cre+).9 In the second model, circulating cells from the donor were labeled with β-galactosidase from the double promoter lacZ/EGFP mouse.9 Transdifferentiation was graded by a yield of β-galactosidase myocytes alone (0.1%), and fused cells would be converted to GFP from the Mer-Cre-Mer recipient (Cre-based excision of lacZ; 10.1%).9 For the second model, the time line was extended by an additional 2 weeks to allow for tamoxifen–induced Cre-based recombination (Figure).9 These 2 models support the idea that established circulation yields much higher rates of fusion between BMCs and cardiomyocytes (50–100 fold) relative to direct transdifferentiation events in the myocardium, which is still above the negligible events reported by Balsam et al.8 Cre-based recombination is not 100% efficient, which may limit the interpretation of new cardiomyocytes through transdifferentiation in the second model.11,12 However, similar percentages obtained by 2 separate parabiosis experiments, with and without tamoxifen treatment, indicate that Cre-based excision was not a significant issue in confirming the frequency of circulating cell commitment and fusion.

The existence of heterogeneous cell populations, such as HSCs, mature lineage+ cells, and mesenchymal stem cells, allows for broad application of bone marrow–derived cells. Resident BMCs are reported to express early cardiac transcription factors Nkx2.5 and GATA4, but this endogenous cardiac precursor population decreases with the age of the mouse.13
Although the majority of circulating cells were confirmed to be CD45\(^+\), cardiomyogenic precursors from the bone marrow could be responsible for the small number of transdifferentiation events reported.\(^9\) The presence of GFP\(^+\) endothelial and smooth muscle cells was not validated to arise from cellular commitment or fusion events, but the data suggest in part that circulating cells have significant vascular potential. In reference to transplantation studies, ex vivo expansion of HSCs may favorably extract and propagate cardiac and vascular precursors from the bone marrow to promote increased transdifferentiation events seen in initial therapy models after injury.\(^1,2\)

The process of hematopoietic cell fusion has been observed in the brain, liver, and heart.\(^14-16\) BMCs directly fuse to apoptotic cardiomyocytes, a homeostatic process increased in tissues exposed to stress.\(^17,18\) In addition, myoblast fusion, a process of skeletal muscle differentiation and regeneration, is initiated by the externalization of the phosphatidylserine on the cell surface.\(^9\) To confirm that cross-circulation is needed for observable cell fusion within the heart, parabiosis surgery was initiated for 3 or 5 days before MI or at the time of MI relative to studies analyzed after 1 month of parabiosis.\(^9\) Incomplete cross-circulation (day 3 and day 5) in the recipient yielded far fewer fusion events (6%–7%) and decreased to \(\approx 1\%) of fused cardiomyocytes at the time of surgery and after a 4-week follow-up.\(^9\) In the discussion, Balsam et al\(^9\) suggest that cross-circulation requires at least 7 days, but parabiosis was performed after MI injury which may have limited the occurrence of cell transdifferentiation. Although fusion-based events were not quantitated in the original study, the proposed change in the experimental protocol supports the data of hematopoietic cell–based reprogramming.\(^9\)

Myocardial injury transiently upregulates inflammation and amplifies cellular proliferation and death, leading to increases in cell permeability and susceptibility to cell fusion. Additional surgery after MI may have deviated innate inflammation to the parabiosis surgery site, limiting bone marrow migration to the myocardium.\(^8\) In contrast to adoptive transfer studies, fusion seems to be the preferred mechanism of migrating BMC persistence and engraftment under acute pathological settings proposed in the current model.\(^9\) Identifying the population(s) within the cohort of circulating cells that preferentially choose fusion over transdifferentiation events in damaged tissue should be considered in future investigations. BMCs display enhanced paracrine abilities, a main mechanism for cardiac progenitor recruitment and myocardial repair after direct injection into the damaged heart.\(^11\) BMCs fused to mature somatic cell types confer properties, such as proliferation and multipotency, by movement of transactivating factors.\(^20-22\) Cellular reprogramming of cardiomyocytes after fusion with BMCs may support survival and protective signaling in combination with a beneficial paracrine milieu to support sustained myocardial repair.

The data presented by Wu et al\(^9\) are not to highlight the inherent rigidness of hematopoietic cells. It is well established that HSCs easily derive mature blood cells validating successful bone marrow reconstitution assays in model organisms. The conversion of circulating cells to cardiomyocytes reported is not exceptional, but the frequency is markedly increased relative to initial results.\(^4\) The data presented support the qualitative changes of circulating cells to form striated and mature cardiomyocytes albeit in limited quantities.\(^5\) Transdifferentiation, also known as lineage reprogramming, may occur rarely, but it remains a fascinating process. Focusing on the mechanisms that support transdifferentiation over cell fusion would be a much needed advancement to support the use of a variety of bone marrow–derived cells for cellular therapy. Similar to the controversies in the cardiac stem cell field, this study reopens the question as to the plasticity of noncardiac cells to contribute to the myocardium during acute injury stimulus. However, would the same level of contribution arise in a chronic diseased model? In general, are we to think that the functional capacity of hematopoietic cells is nonexistent or insignificant, or have we found the ideal adult stem cell population that has been tarnished by a long-standing stigma for myocardial repair? Studies like this, which involve evaluating the inherent biology of stem cells, should be performed initially before concluding the relevance of a cell population that has potential promise for clinical use.

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M.A. Sussman is a founder and co-owner of CardioCreate Inc. P. Quijada reports no conflicts.
References


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