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EDITORIAL COMMENT

Boot Camp for Mesenchymal Stem Cells*

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Stem cell transplantation is a promising new treatment for ischemic cardiomyopathy, offering the unique opportunity for true cardiac repair and regeneration. Several different types of stem and progenitor cells are being explored for this purpose (1). Among the various cell types, mesenchymal stem cells (MSCs) (alternatively named multipotent stromal cells) are potentially attractive, as they are easily isolated and expanded, and they exhibit low immunogenicity, rendering allogeneic applications plausible (2). Despite their potential benefits, results in animal models and in humans have been variable, and little is known regarding their mechanism of action. Are all MSCs created equal? Can they really regenerate heart tissue directly? If so, is direct cardiomyogenesis required for a therapeutic effect?

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Mesenchymal stem cells, first described by Friedenstein in 1961 (3), are self-renewing precursors of nonhematopoietic stromal tissues characterized by: 1) adherence to plastic in culture; 2) surface expression of CD105, CD90, and CD73; 3) lack of expression of hematopoietic markers; and 4) the capacity to differentiate into fibroblasts, osteoblasts, adipocytes, and chondroblasts under specific in vitro conditions (4). Originally, MSCs were isolated from the bone marrow, but similar populations have been reported in several other tissues including the heart (5). In vivo, MSCs typically reside in perivascular niches (6) where they create/function as a stromal network, supporting other cell types (e.g., hematopoietic cells in bone marrow) (7) and contributing to the creation and maintenance of connective tissues. Although traditional isolation of MSCs by plastic adherence results in notoriously heterogeneous preparations with respect to cell size, morphology, proliferative capacity, and potential for differentiation (8), it is commonly accepted that these cells compose a multipotent adult stem cell population, but their capacity to differentiate into excitable tissues is not well-established (2).

The ability of MSCs to undergo true cardiomyogenic transdifferentiation, in particular, remains highly controversial (9,10). In vitro, MSCs can express cardiac-specific proteins but do not display the typical electrical properties of true cardiomyocytes (10). In vivo, there is immunohistologic evidence for low cardiac engraftment and transdifferentiation after MSC transplantation (11), although such findings are not universal (12), even within the same laboratory (13). In order to boost aptitude for cardiomyogenic differentiation, different strategies of MSC ex vivo manipulation have been employed with various degrees of success, including exposure of cells to the deoxyribonucleic acid demethylating agent 5-azacytidine, pre-treatment with growth factors, hypoxic pre-conditioning, and genetic engineering (2). However, there is still a lack of convincing evidence that MSCs can differentiate into functional cardiomyocytes.

In this issue of the *Journal*, landmark work by Behfar et al. (14) investigates the feasibility of deriving cardiomyocytes from human mesenchymal stem cells (hMSCs) through mimicry of natural/embryonic cardiogenic signaling. Bone marrow-derived hMSCs were isolated from patients undergoing coronary artery bypass surgery. In their naive state, hMSCs exhibited poor capacity for cardiomyogenic differentiation in vitro as well as limited potential for myocardial repair in vivo. However, ex vivo priming of cells with a cardiogenic cocktail of growth factors (consisting of transforming growth factor-beta$_1$, bone morphogenetic protein-4, activin-A, retinoic acid, insulin-like growth factor-1, fibroblast growth factor-2, alpha-thrombin, and interleukin-6) up-regulated cytosolic expression and promoted nuclear translocation of cardiac transcription factors, successfully converting weakling hMSCs into ones capable of strong cardiopoiesis. Importantly, this "boot camp" strategy resulted in a dramatic improvement of functional and structural end points following intramyocardial injection into nude mice with ischemic cardiomyopathy. The investigators provide the first convincing evidence that MSCs, at least in vitro, can in fact become functional cardiomyocytes, exhibiting sarcomerogenesis, mitochondrial maturation, and electromechanical coupling. Using a cocktail-based approach (previously employed by the same group to stimulate cardiopoiesis of embryonic stem cells) (15) while avoiding co-culture of MSCs with other cell types, the capacity of bone marrow-derived hMSCs to undergo cardiomyogenic transdifferentiation was convincingly established and distinguished from fusion phenomena.

Behfar et al. (14) further noted that naive MSCs demonstrated vast interpatient heterogeneity in terms of their aptitude for in vitro cardiomyogenic transdifferentiation and potential for myocardial repair. Indeed, only 2 of 11

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individuals yielded hMSCs with robust expression of cardiac transcription factors and the ability to boost ejection fraction in injured mouse hearts, but none of the clinical characteristics was predictive of the reparative cytotype. In contrast to some previous reports (11,12), but confirming others (16), treatment with naive hMSCs did not improve cardiac function relative to saline-treated controls.

Because MSCs can be isolated from a variety of different tissues, including the heart (5,17), tissue of origin might be of particular importance. It seems plausible that cells may already be primed toward differentiation along lineages specific to tissues in which they reside. Consistent with this idea, the molecular profile, differentiation potential, and function can vary widely among MSC preparations depending on their origin (18,19). If tissue source proves to be important, heart-derived MSCs merit particular investigation, as they may be more predisposed to cardiomyogenesis than are bone marrow MSCs. They may also possess a specialized ability to support cardiac progenitor cells (CPCs) in the heart, just as bone marrow MSCs physiologically support hematopoietic cells in the marrow.

A final notable aspect of the present study (14) is that a MSC-derived cardiopoietic cell phenotype (regardless of whether it was observed spontaneously in rare individuals or as a result of guided cardiopoiesis) was associated with a dramatic increase in reparative efficacy, when compared with noncardiopoietic MSCs. This increase was attributed to more robust direct (cardiomyogenesis, angiogenesis) and indirect (cardiomyocyte cell cycle re-entry, endogenous stem cell recruitment) contributions to infarct repair by conditioned cells compared to naive MSCs (Fig. 1). To date, the majority of in vivo studies have demonstrated at least modest functional improvement following MSC transplantation, despite undetectable to low levels of long-term engraftment and differentiation (12,20). This implies that MSCs exert their beneficial effects mainly through indirect paracrine actions rather than by contributing directly to tissue regeneration (2,21). Importantly, the fact that significant long-term engraftment is not required for functional benefit (20), together with the purportedly low immunogenicity (22) of MSCs, support the notion that allogeneic transplantation without immunosuppression may be feasible. On the other hand, the positive correlation between MSC engraftment and functional recovery in post-ischemic cardiomyopathy suggests that some of the benefit may be due to long-term engraftment and trilineage differentiation of MSCs (11), even if the absolute survival of transplanted cells is low.

A particularly important function of cardiac MSCs may be to enhance the survival and/or potency of cotransplanted CPCs in cardiomyoplasty. We have been investigating cardiosphere-derived cells (CDCs) grown from percutaneous endomyocardial biopsies for human therapeutic applications. Cardiosphere-derived cells are a natural mixture of heart-derived cell subpopulations, including true CPCs (c-kit$^+$/CD90$^-$) as well as cardiac MSCs (c-kit$^-$/CD90$^+$) (23,24). One logical approach to cardiomyoplasty is the selective purification, expansion, and injection of CPCs (25,26). We find, however, that CDCs outperform purified CPCs. In experiments with intramyocardial injection of human CDCs in a mouse myocardial infarction model, the spontaneously emerging unselected mixture of CPCs and cardiac MSCs resulted in a higher ejection fraction at 3 weeks than either purified c-kit$^+$ or CD90$^+$ cells from the same source (27). These findings hint that cardiac MSCs help CPCs to engraft and/or function, presumably via synergistic paracrine actions as well as direct myocardial regeneration (28).

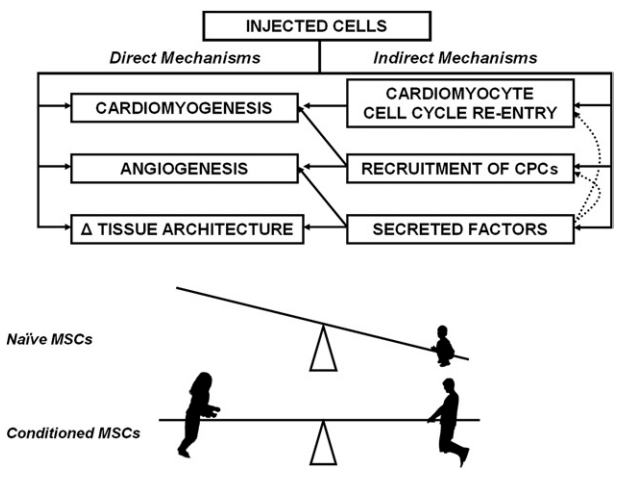

Figure 1 **Schematic Depiction of the Balance of Direct Versus Indirect Mechanisms Underlying the Salutary Effects of MSCs**

Schematic depiction of the balance of direct (cardiomyogenesis, angiogenesis, and favorable changes in tissue architecture) versus indirect (cell cycle re-entry, recruitment of cardiac progenitor cells and secreted factors) mechanisms underlying the salutary effects of mesenchymal stem cells (MSCs). Indirect mechanisms predominate in naive MSCs, but the benefit is small: a child tips the seesaw. Boot camp-conditioned MSCs exhibit balanced, robust mechanisms, akin to the interplay of 2 physically-fit adults.

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Key Words: ischemic cardiomyopathy ■ transplantation ■ patient-derived ■ cell therapy ■ heart failure.