MOLECULAR THERAPY
The Journal of the American Society of Gene Therapy

Unraveling the Mechanistic Basis of Mesenchymal Stem Cell Activity in the Heart

Heart failure affects millions of Americans each year. Although many diverse factors can contribute to its etiology, cardiomyocyte loss is frequently involved. Most studies indicate that the ability of the myocardium to regenerate in the setting of heart disease is limited; consequently cardiomyocyte loss is cumulative and if unchecked will ultimately lead to organ failure. Interventions aimed at blocking or reducing cardiomyocyte death in response to acute or chronic insult are thus of considerable therapeutic value. In addition, several strategies to augment cardiomyocyte number have been proposed, including induction of cardiomyocyte cell cycle activity as well as transplantation of cardiomyocytes or cardiomyogenic stem cells. These latter strategies are based on the notion that increasing cardiomyocyte number in diseased hearts will result in a concomitant increase in cardiac function, provided that the nascent cells participate in a functional syncytium with the rest of the heart.

Mesenchymal stem cell (MSC) transplantation has been extensively studied in the setting of experimental myocardial injury [1,2]. Although many studies demonstrated that MSC transplantation enhanced cardiac function in animals with experimental infarcts, the mechanistic basis for functional improvement was somewhat controversial. Several recent reports have significantly clarified the situation. An important breakthrough came from the Dzau laboratory in 2003. These investigators demonstrated that, when transplanted into rat hearts following permanent coronary artery occlusion, MSCs expressing a transgene encoding Akt (a serine threonine kinase with potent pro-survival activity) exhibited greater retention as compared to MSCs lacking the transgene [3]. Mice with Akt-expressing MSCs exhibited a dramatic reduction in acute infarct size and acute cardiomyocyte apoptosis, as well as an increase in cardiac function at two weeks post-injury. Interestingly, MSCs lacking the Akt transgene also had a beneficial impact, but to a lesser degree. Immune histology suggested cardiomyogenic differentiation of the MSCs. In subsequent studies [4,5], Dzau and colleagues demonstrated that conditioned medium prepared from Akt-expressing MSCs cultured under hypoxic conditions essentially replicated the results observed with MSC transplantation. These data suggested that the MSCs acted through a paracrine mechanism, and questioned the relevance of the apparent cardiomyogenic differentiation reported in previous studies.

The most recent installment of this series, which appears in this issue of *Molecular Therapy* [6], further examined cardiomyogenic differentiation of transplanted MSCs. In this study, Noiseux and colleagues injected MSCs expressing EGFP, Cre recombinase and Akt into the hearts of R26R mice following permanent coronary artery occlusion. R26R mice harbor a Cre-dependent β-galactosidase reporter in the ubiquitously-expressed *Rosa26* locus. In this system, EGFP activity can be used to track donor-cell survival, and induction of β-galactosidase activity can be used to track fusion events between donor and host cells. Donor MSCs were readily detected at 3 and 7 days following transplantation, but were only infrequently observed by 14 days post-injury. The vast majority of donor cells did not exhibit a cardiomyocyte phenotype (based on colocalization of EGFP and α-sarcomeric actin immune reactivity). However, a small number of donor-derived cells exhibited a cardiomyocyte-like morphology (only nine cells were detected in over 600 tissue sections examined); these cells also exhibited β-galactosidase activity, indicating that they arose from MSC-cardiomyocyte fusion events. A single cell with EGFP and α-sarcomeric actin immune reactivity, but lacking β-galactosidase activity, was observed. Although this cell may have arisen as a consequence of cardiomyogenic differentiation, it could also have represented a false-positive event if the penetrance of the R26R reporter was less than 100%. Other analyses suggested that fusion events also occurred between the donor MSCs and non-cardiomyocytes.

Collectively, these studies provide considerable insight as to how MSC transplantation impacts function in injured hearts. Clearly, the benefit of MSC transplantation in injured

hearts resides in their ability to secrete cardioprotective paracrine factors in the myocardium, and is independent of their differentiation into cardiomyocytes. Moreover, a hypoxic environment appears to enhance the production of MSC-derived cardioprotective factors. This latter finding might underlie the observation that acute myocardial infarction patients with more severe cardiac dysfunction (and, presumably, a larger ischemic burden) exhibit the greatest benefit from intracoronary infusion of marrow-derived cells [7]. Although enhancement of short-term MSC survival (via Akt expression) increases their cardioprotective activity in experimental animals, long-term MSC persistence is not required for a long-term impact on cardiac function. Similarly, improvement in cardiac function following intra-coronary bone marrow cell infusion in patients with acute myocardial infarction persists for 18 months [8]. However, the observation that cardiac function in patients not receiving intra-coronary infusions "catches up" to that seen in the treated patients makes it difficult to compare the experimental and clinical studies.

While one can easily envision how the transient presence of MSCs could have a long-term impact in the setting of reperfusion injury, it is intriguing that the current study demonstrated a long term impact in the setting of permanent coronary artery occlusion (as opposed to reperfusion injury), particularly given the absence of enhanced peri-infarct vessel density in hearts receiving MSCs vs. controls [5, 9-11]. The mechanistic basis for the sustained enhancement of cardiac function is not fully understood. It may result from the salvage of at-risk cardiomyocytes at the infarct border zone; indeed, previous studies have shown a critical relationship between infarct size and the propensity to progress to heart failure in rats [12]. Alternatively, MSC transplantation may result in more favorable post-infarction remodeling, which in turn may contribute to the sustained impact on cardiac function. It has recently been shown that MSC transplantation results in enhanced cellularity in the infarct scar, which is accompanied by improved tissue elasticity [9]. These characteristics would likely result in increased myocardial compliance as well as reduced scar expansion at the infarct border zone. A similar mechanism likely contributes to the improved cardiac function observed following transplantation of other cell types in injured hearts [13].

Although we are beginning to understand the mechanistic basis for MSC-based cardioprotection, many questions remain. Additional experiments to more thoroughly characterize post-injury remodeling are required to further clarify the mechanisms by which exogenous MSC transplantation enhance cardiac function in injured hearts.

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