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EDITORIAL COMMENT

In Vitro Adherence Defines Therapeutic Cardiac Mesenchymal Cell Subpopulation*

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Heart failure (HF) remains one of the greatest growing human health threats with global penetrance, affecting an estimated 5.7 million adults 20 years and older in the United States alone, a figure that could increase 46% and top 8 million by 2030 (1). Contributing to the HF prevalence rate is the continuing high incidence of myocardial infarction (MI). Annually, more than 1.1 million patients are discharged from American hospitals with a diagnosis of acute coronary syndrome (1).

Ischemic cardiomyopathy is a major contributor to HF, with massive numbers of cardiomyocytes lost as a result of MI. Ongoing apoptosis post infarction is a significant contributor to HF progression, exacerbating the loss of viable myocardium. With the discovery that cardiac regeneration can occur, cell therapy emerged as a novel and safe approach to cardiac repair and regeneration; however, for regeneration to have meaningful benefit, complex post-delivery molecular- and cellular-driven events must take place. These events include chemotaxis, proliferation, differentiation, and integration at the cellular level, as well as reverse remodeling and restoration of function throughout the myocardium.

Historically, cardiac regeneration research has focused on delivering stem cells of mesenchymal and hematopoietic origin to the heart with the goal of regenerating cardiomyocytes lost during either MI or progression to HF. Myocardial residence times of delivered cells have been shown to be discouragingly short. Therefore, their direct homing, adhesion, proliferation, and differentiation to functioning myocardium is of extremely limited benefit, which means improved outcomes must be from alternative mechanisms. Lineage-tracing studies have documented minimal cardiac stem cell (CSC) cardiomyogenesis, shedding doubt that they mature into functioning cardiomyocytes in the adult mammalian heart, although controversies continue (2). Optimism remains high for several reasons:

1. Stem cells express beneficial paracrine factors that influence endogenous stem cells;
2. Endogenous resident stem cells in the adult heart have cardiomyogenic potential (3);
3. cKit$^+$ resident CSCs have multiple lineage potential;
4. Mesenchymal stem cell or CSC delivery to the failing heart results in improved cardiac function, angiogenesis, and detection of populations of cells expressing both CSC and cardiomyocyte markers (4);
5. Although extremely rare, cardiomyocyte proliferation has been demonstrated by cKit cell fusion with cardiomyocytes in the failing heart (5), as well as in hypoxic healthy or failing hearts (6). These findings supported the hypothesis that paracrine factors are the main effectors of endogenous CSC responses in the failing heart, leading to cardiac repair with inconsequential levels of cardiomyogenesis. Furthermore, regardless of mechanism, the relative therapeutic potential of the various stem cells is unclear. Thus, more complete comparative studies focusing on stem cell therapeutic efficacy, cell signaling in the heart, and the effects on cardiac cell survival, remodeling, and function in the setting of HF remain unmet needs.

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Recently, Wysoczynski et al. identified a cKit$^+$ stable subset of progenitors isolated from the murine heart that can be functionally separated from other stem cells based on the kinetics of adherence to culture plates and bottles (7). Now, in this issue of the *Journal*, they show that subsets of cardiac mesenchymal cells (CMCs) have contrasting molecular phenotypes that include signaling factors and contrasting efficacies in the setting of ischemic cardiomyopathy (8).

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Under the experimental protocols used, CMCs that adhered to plastic plates within 4 h were less therapeutically active than CMCs that adhered over at least a 24-h period. Therefore, a functional phenotype of slowly adhering (SA) CMCs was defined, and this property could be used to enrich more beneficial subpopulations. In comparative studies in the setting of ischemic cardiomyopathy, cKit-sorted SA CMCs stimulated angiogenesis in the infarcted heart, whereas rapidly adhering CMCs uniquely elevated collagen deposition and hypertrophy endpoints. It is notable that although acute and subacute fibrosis is beneficial for stabilizing the ventricular wall, there remains the potential for this progenitor cell population to stimulate pathophysiological chronic scar expansion. The distinctive pattern of cytokine gene expression by SA CMCs included increased expression of proproliferative and prosurvival factors and reduced expression of proinflammatory and profibrotic factors relative to CMCs. These contrasts in expression profiles were consistent with improved structural and functional outcomes driven from SA CMC delivery to mice with ischemic cardiomyopathy. These results highlighted the fact that stem cells, regardless of source, are heterogeneous collections of cells with heterogeneous therapeutic potential and are highly responsive to their environments. Therefore, detailed molecular and functional characterizations of mesenchymal stem cells, CSCs, and CMCs and their subtypes are needed to define their relative therapeutic potential and improve translation of cell therapy for HF to the clinic.

Some of the most promising therapeutic stem cells have been isolated from the myocardium itself, driving repair via paracrine mechanisms. Wysoczynski et al. (8) describe a subpopulation of CMCs that are relatively easy to purify. The expression of surface markers (and thus phenotype) on SA CMCs appears to be more stable than that of previously reported cKit$^+$ progenitors. SA CMCs are highly suitable for clinical use because the phenotype is more stable and they likely secrete a more consistent suite of paracrine factors. Because these cells impart much of their beneficial effects via a complex suite of soluble and exosomal factors, future complementary approaches include direct delivery of secreted cell products, potentially forgoing the need to deliver the cells themselves. However, the complex compositions of cell secretomes responsible for the multifactorial effects, including modulating immune responses, scar expansion, and apoptosis, as well as improving cardiac function, remain poorly characterized.

The contrasting effects of SA CMCs versus rapidly adhering CMCs highlight the potential for expression of both therapeutic and pathophysiological paracrine factors, which also are poorly understood. Because stem cells are highly sensitive to their environments, it is likely that their secretome compositions change in response to being delivered to the failing heart. Although in the preclinical exploratory phase (9,10), clinical translation of secretome delivery to the failing heart will require more complete characterization of stem cell phenotypes and in vitro compositional control of their secretomes. Until these unmet needs are met, cell delivery remains as one of the most promising approaches to myocardial preservation and repair in the setting of ischemic and nonischemic cardiomyopathy.

Although cell therapy is safe, its full therapeutic potential has yet to be realized. Currently, regeneration of cardiomyocytes in the setting of HF has been especially rare and inconsequential. Despite this current limitation, preservation of viable myocardium and its function, although limiting inflammation and fibrosis, is commonly reported. Wysoczynski et al. are currently identifying and characterizing a subset of cardiac mesenchymal cells that can be readily isolated from murine hearts based on their adherence properties and are therapeutic in the setting of ischemic cardiomyopathy.

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