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EDITORIAL COMMENTARY

Myocardial regeneration for chronic heart failure: Not as easy as it sounds

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Virginie and colleagues¹ describe the absence of functional benefit from injections of human embryonic stem cell-derived (hESC) cardiac progenitors into chronically volume overloaded piglet right ventricles. Further, the authors report 0 persistence of injected cells in the right ventricle myocardium, no neoangiogenesis, no myocardial regeneration, and quantitatively no effect on interstitial myocardial fibrosis. They did report a statistically insignificant reduction in arrhythmogenicity as well as a roughly 50% decrease in perimycocyte fibrosis in the cell-treated animals compared with the sham injected hearts. The significance of this is unclear, because both chronic ventricular overloading and ischemic necrosis result in interstitial fibrosis leading to dysfunction. It is yet another failed animal

FIGURE 1. Immunohistochemistry image of atrioventricular cushions (*short axis*) demonstrating marked periostin expression during atrioventricular valve morphogenesis in human embryonic heart (53d) (4',6-diamidino-2-phenylindole blue stain indicates the nuclei, green stain indicates periostin; 100 $\times$ magnification). Periostin is just 1 of numerous field specific signaling proteins temporally expressed during cardiac development defining different phenotypic destinations for adjacent cells, matrix compaction, and subsequent structural orientations. Many such cues may well be required for therapeutic tissue engineering of replacement cardiac tissues.

experiment exploring a directly administered multipotential cell-based therapy for chronic heart failure. So why is it of interest?

First, the promise of cell/tissue-based regenerative surgical therapies is just so alluring and seemingly simple; and yet, since the invention of the term *tissue engineering* in the late 1980s, such approaches have been shown to be exceedingly complex, frustrating effective clinical translation in part due to critical gaps in the knowledge of relevant cell signaling and cardiac developmental triggers (Figure 1).

Second, the authors have developed a calibrated regurgitation model that recapitulates the progressive RV failure seen in congenital heart disease that is very different from the ischemic-scar-based myocardial failure of coronary disease, the usual target for myocardial regenerative research.

Third, their experimental design is quite good with a control group in which hearts received injections of carrier without cells 4 months after creation of pulmonary valve insufficiency and like the test group, were evaluated after an additional 3 months. Many studies of myocardial regeneration have reported early positive results at 1 month, but further experiments demonstrate no sustained benefits.

Fourth, the hESCs chosen are a fairly well characterized National Institutes of Health-approved line (ie, HUES-24) with a recipe for deriving cardiac progenitor cells using

bone morphogenetic protein 2 signaling, and then subselected for MESP-1⁺, which is regarded as the “master regulator” of early generation cardiac mesoderm² and SSEA-1⁺, which in humans is upregulated early in cardiac progenitor cell differentiation.³

Fifth, the use of immunosuppressed pigs to test hESCs demonstrates the difficulty in analyzing the results due to the anti-inflammatory effects of locally injected hydrocortisone and postoperative tacrolimus. Xenotransplant antigenicity mitigates the utility of large animal models for testing human cells.

So what can we take away from such a “negative” study? A lot really. Because the most promising results with lineage specific so-called stem cells derived from autologous bone marrow, hESCs, or induced pluripotent stem cells have been in subhuman primates and human clinical studies,^{4,5} funding sources need to be forewarned that translational progress in myocardial regeneration will likely require expensive animal modeling in subhuman primates or alpha-1,3-galactosyltransferase knockout pigs. The heart failure of chronic volume and pressure overloading in congenital disease is very different than the ischemic cell necrosis and regional scarring from adult coronary disease and must be explored with appropriate and specific models. The tissue engineering needed to replace absent or damaged structures (eg, bone, trachea, heart valves, or tendons) is different than restoration of functional cell populations in damaged organs (eg, hepatocytes, islet cells, and myocytes) as in the former, the scaffolds can be optimized. Exogenous cell-based therapies for myocardial regeneration/rejuvenation may not actually be required because results suggest benefits appear to be via paracrine mechanisms independent of the survival of injected therapeutic cells. Scaffold properties of a normal fibrous cardiac skeleton as well as cell signaling from other cell types in residence are likely critical cues. Thus, just mechanically placing multipotential cells where they “ought to be” in a pathologic substrate is unlikely to succeed without further modulation of the myocardial niche. Small, large molecule, or gene therapy approaches may actually be more effective and sustainable, because these could target autologous repair mechanisms. Mechanisms by which laudable inflammation⁶ enhances homing of autologous tissue-based multipotential repair cells need to be extensively explored for critical therapeutic paracrine niche cues that are currently so elusive; for example, by targeting M1-M2 macrophage phenotype polarization, a spectrum of inflammatory responses where M2 is a tissue repair response characterized by anti-inflammatory cytokines (eg, interleukin-10) and M1 is a more classic inflammatory “killer” type characterized by TNF- α and interleukin-6 signaling. Macrophages are critical to regenerative mechanisms: macrophage depletion results in failure of salamander limb regeneration.⁷ Just as direct myocardial injections of vascular endothelial growth factor

failed to drive therapeutic angiogenesis, it is also likely that injecting whatever the fashionable multipotential cell of the moment might be (eg, induced pluripotent stem cells, hESCs, and bone-marrow-derived mesenchymal stem cells) is an approach too simplistic to succeed, because it does not address the overall microenvironment nor integrate temporally into programmable tissue regeneration pathways.⁸

We hope the dream of therapeutic myocardial regeneration is not abandoned but such “failures” are used as guides to creative approaches. Historically, once the applicable biological rules are understood, the subsequent therapies yielded are often surprisingly straightforward and elegant.

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