See Article page 975.

Commentary: Delivering the cargo...

S. Ram Kumar, MD, PhD, FACS

The mammalian cardiomyocyte is a specialized cell with the unique capability of sustaining contractile function throughout the lifetime of an individual. However, this highly differentiated cell type has limited proliferative capacity, in contrast to the cardiomyocyte of lower vertebrates such as zebrafish or newt, which maintain robust regenerative potential.¹ As a consequence, loss of cardiomyocytes due to myocardial injury or pressure overload in humans results in the formation of a fibrotic scar that is incapable of contributing to cardiac contractility, leading to progressive reduction in heart function and, ultimately, heart failure. As the field of molecular biology advanced, novel biological therapies to address this vexing problem of end-stage heart failure began to emerge.² The most mature of these efforts introduces exogenous stem or “stem-like” cells into the injured myocardium. Several animal and human studies have shown significant improvements in heart function after cell-based therapies in the context of both ischemic myocardial damage³ and single-ventricle heart disease.^{2,4} However, detailed analyses have failed to demonstrate that the injected cells reliably engraft or produce new cardiomyocytes.⁵ More recently, murine studies have revealed that as mice transition from embryonic to postnatal environment, cardiomyocytes transiently retain their proliferative capability in the neonatal period.⁶ During this time, the majority of mononuclear diploid cardiomyocytes present at birth transitions to a multinucleated or polyploid mononuclear state, which is associated with reduced regenerative capacity. The frequency of mononuclear diploid cardiomyocytes in the adult heart correlates with the animal’s ability to regenerate and recover from ischemic injury.⁷ Based on this evidence, it is now hypothesized that the functional

CENTRAL MESSAGE

Precise understanding of the underlying molecular mechanisms will help bring the promise of cardiac biologic therapy into the realm of clinical reality.

improvement observed after cell transplantation into injured hearts is likely related to paracrine effects that alter the microenvironment or induce proliferation of resident proliferation-competent cardiomyocytes.

The exact nature of the molecule(s) that orchestrate these purported paracrine effects, however, continues to be elusive. It is not known whether this molecule is a single protein or nucleic acid or a combination of several different molecules. This molecule could be secreted into the extracellular space by the injected cells in its simplest form or be trafficked via elaborate intercellular communication machinery. Our understanding of exosomes is particularly relevant for this latter mechanism. Multivesicular bodies (MVBs), which are members of the endosomal compartment of cells, carry cargo wrapped in intraluminal vesicles (Figure 1). When these MVBs fuse with the cell membrane, they release their cargo into the extracellular milieu as vesicles, termed “exosomes.” Exosomes have been known to carry a plethora of molecules, including DNA, RNA, microRNA, lipids, soluble and membrane-bound proteins, and so forth. Exosomes can regulate immune responses,⁸ with particular significance in viral infection and cancers. Given their effective role in transferring macromolecular cargo between cells, exosomes are attractive candidate mediators of the paracrine effects of transplanted cells during cardiac regeneration.⁹ If indeed exosomes are necessary for the observed beneficial effect of cellular therapy, are they also sufficient? In this issue of the *Journal*, Bittle and colleagues¹⁰ demonstrate in a preclinical juvenile porcine model that injection

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FIGURE 1. Schematic representation of exosomes. Endocytosed material and cytoplasmic materials are organized in the endosomal compartment of the donor (exosome-generating) cell, leading to the formation of an MVB that contains many intraluminal vesicles. Some MVBs fuse with lysosomes leading to their degradation. Others fuse with the plasma membrane secreting their vesicles into the extracellular environment as exosomes. These exosomes carry cargo such as DNA, RNA, micro-RNA, lipids, and membrane-bound and soluble proteins. They transmit signals to target (recipient) cells by attaching to the cell surface or after endocytosis into the recipient cell. ER, Endoplasmic reticulum.

of exosomes isolated from cardiophere-derived cells into the right ventricular (RV) myocardium after acute pressure overload effectively attenuates RV dysfunction. As alluded to by the authors, such acellular preparations offer a novel therapeutic approach to myocardial regeneration, which may have significant benefits compared with whole cell transplantation. First, the manufacturing, storage, and transportation of exosomes are far less cumbersome compared with live cells. Second, exosomes are highly efficient vehicles to deliver therapeutic molecules. Unlike synthetic polymers, membrane-bound exosomes are better received by living cells. Exosomes can carry molecules like microRNA that are, by themselves, unstable in the extracellular environment. And, it is believed that exosomes exhibit target cell specificity, avoiding adverse off-target consequences. Last, exosomes can be isolated from a variety of “stem-like” cells, thereby ensuring a stable and diverse source. Although the authors of the current work¹⁰ used 3 different exosome preparations isolated from cardiophere-derived cells, exosomes can also be isolated from other cardiac progenitor cell cultures, mesenchymal stem cells, and umbilical cord blood-derived mononuclear cells, to name a few.

Our enthusiasm is dampened by the reality that the current work does not shed light on the mechanism by which the exosomes exert their effect. The nature and contents of the exosomes have not been characterized, which precludes any understanding of physiologically relevant molecules. Other than a reduction in cardiomyocyte hypertrophy, no other significant change, in particular cardiomyocyte proliferation, was demonstrated in treated hearts. Although exosomes are generally less immunogenic, XO-1, which

resulted in best functional recovery, also induced the highest degree of inflammation and elevation in the inflammatory marker, erythrocyte sedimentation rate, which is intriguing. As the authors concede, their model is substantially different from the systemic RV of patients with single-ventricle physiology, making extrapolation difficult. So, like any good scientific study, the work by Bittle and colleagues¹⁰ raises more questions than it answers. Continued rigorous evaluation of the mechanisms by which biological cardiac regenerative therapies function is critical to ensure that the promise of molecular medicine enters the realm of clinical reality.

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See Article page 975.

Commentary: An idea with some muscle behind it

Danielle Gottlieb Sen, MS, MD, MPH, and
Bret A. Mettler, MD

Great ideas originate in the muscles.

—Attributed to Thomas A. Edison (1847-1931)

Six of nine species of cold-blooded animals have the capacity to regenerate their hearts during adult life. And even though adult human cardiomyocytes exhibit a nonregenerative response to injury, and subsequent systolic heart failure, many fetal mammals retain a regenerative response and demonstrate cardiomyocyte replication.¹ For those interested in treating systolic heart failure in children, these encouraging findings have fueled an effort to induce children's hearts to regenerate.

Of specific interest to congenital heart surgeons is the fragile group with single right ventricles (RV), whose outcomes are highly dependent on systolic function of the systemic RV. RV failure is addressed with the limited-supply solution of transplantation, and patients accumulate morbidities and compromised quality of life while awaiting a heart.

The view that the heart muscle cannot regenerate was upended with the discovery that bone marrow-derived stem cells could be utilized to regenerate heart muscle.

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CENTRAL MESSAGE

Regenerative medicine, including stem cell-based therapies aimed at improving myocardial function, is advancing incrementally.

As scientists have established methods for the manipulation of stem cells, tissue-derived mesenchymal stem cells have emerged as an exploitable source of regenerative potential. Further insights into inducible pluripotent stem cells have led to differentiation and maturation techniques that allow the enrichment of cardiomyocyte progenitor populations. The field has subsequently experimented with multiple lineages of stem cells (bone marrow-derived stem cells, umbilical cord-derived stem cells, myocardial resident cardiac progenitor cells, and cardiosphere-derived progenitor cells) and, more recently, the secreted soup of incompletely understood products of cardiosphere-derived cell differentiation known as exosomes.²

Clinical trials have been completed in the field to date with 2 main delivery methods to the myocardium and variability in the timing of intervention, to approach the question of whether there are cell or cell-derived therapies that can ultimately change outcomes in univentricular hearts. Most trials have demonstrated modest improvements in right ventricular fractional area change, between 5% and 10%, through the duration of the trial.