Endothelial Cardiac Cell Therapy
Large-Animal Studies and the Elephant in the Room

Wei-Yu Chen, Richard T. Lee

Cell-based therapies represent a promising strategy for regeneration of myocardial tissue, and it is clear that impaired contractile function can be improved even when transplanted cells do not persist in the myocardium. Many cell types and mechanisms have been proposed for the benefits of cardiac cell therapy, including the formation of new myocytes, endothelial cells, and vascular smooth muscle cells, as well as through paracrine effects. In part because we do not yet understand these mechanisms, the ideal cell type and delivery approach for cardiac cell therapy has not yet emerged.

Endothelial progenitor cells (EPCs) are a subset of hematopoietic cells found in the bone marrow that have the potential to differentiate into endothelial cells and have a role in promoting angiogenesis and prosurvival signals to cardiomyocytes. EPCs are readily isolated from the blood and the bone marrow, and clinical studies suggest that cell-based therapy with EPCs can improve myocardial function. A subset of mesenchymal stem cells can differentiate into cardiomyocytes under specific conditions in vitro. Bone marrow–derived cell therapy can stimulate endogenous cardiomyocyte progenitors and promotes cardiac repair. Methods to limit teratoma formation include genetic selection of differentiated embryonic stem (ES) cells, or differentiation of ES cells in vitro into cardiomyocytes or endothelial cells before injection. An inherent difficulty in controlling the growth and differentiation of ES cells and other pluripotent stem cells is that the timing with which specific signaling pathways are activated might be crucial. Until now, clinical trials have used cell types that are readily available (bone marrow mononuclear cells and EPCs), but these cell types do not necessarily reflect stem cell populations that are most likely to regenerate myocardium.

Growth factor and/or cytokine release by injected cells is frequently suggested as a potential mechanism of action of cardiac cell therapy. Local intramyocardial delivery of cytokines or growth factors could be more reproducible than injection of heterogeneous populations of stem cells or progenitor cells. Although attempts have been made to identify paracrine mediators and enhance paracrine effects, no single unifying paracrine mediator has been identified.

In this issue of Circulation Research, Gu et al provide a significant step forward in the biology of cell-based therapy for myocardial infarction. They demonstrate that porcine induced pluripotent stem cell–derived endothelial cells (piPSC-ECs) improve myocardial function through paracrine activation in a murine myocardial infarction model. Using a modified protocol for the derivation of human endothelial cells from iPSCs, they successfully generated endothelial cells from porcine iPSCs that shared similar morphological and functional properties as endothelial cells from the aorta. They generated porcine iPSCs (piPSCs) from adipose stromal cells using lentivirus carrying Oct4, Sox2, Klf4, and c-Myc and confirmed the pluripotency of the piPSCs by in vitro and in vivo differentiation assays. They then characterized the endothelial cells derived from the piPSCs by using the in vivo Matrigel plug assay, in vitro assays for CD31 expression, and capillary tubule formation (Figure).

Gu et al further showed that delivery of these iPSC-ECs into mice after myocardial infarction resulted in significant improvement in ejection fraction 4 weeks after transplantation. In vitro hypoxia showed that piPSC-ECs secreted more proangiogenic cytokines than control. These studies are consistent with the concept of cardiac cell therapy improving cardiac function through paracrine activation. Furthermore, by using a novel microfluidic PCR technique to determine gene expression at the single cell level, they discovered that piPSC-ECs are capable of releasing proangiogenic and antiapoptotic factors in the ischemic environment. These paracrine factors promoted the formation of new blood vessels in the peri-infarct area. Interestingly, the transplanted piPSC-ECs stimulated endogenous angiogenesis, whereas the piPSC-ECs did not form vasculature in vivo. They also demonstrated that the pattern of paracrine release varied among different cell subpopulations. In addition, the authors show the feasibility of tracking the fate of implanted piPSCs in a porcine model using clinical PET/CT, multi-modality MRI, and histology by directly labeling of cells with [18F]-FDG, iron particles, and carbocyanine dye. From a clinical perspective, the findings presented by Gu et al point toward iPSC-derived ECs as a therapeutic strategy to improve cardiac remodeling and function of the heart after myocardial infarction.

The findings of Gu et al raise other intriguing possibilities and important questions. Do the implanted iPSC-ECs recruit endogenous stem cells or vascular progenitor cells from cardiac, bone marrow, or extracardiac vascular stem cell niche? Will the implanted iPSC-ECs and their beneficial effect persist for months or years after implantation, without eventually forming tumors or undesirable cells types such as bone tissues? Although several groups have created iPSCs from...
porcine adult tissue,13,14 generation of a therapeutic cell type from these large animals has remained challenging. Further studies are required to address these unanswered questions.

iPSCs have been created from adult somatic cells of many small and large animals including mice,15 rats,16 monkeys,17 dogs,18 pigs,13,14 and humans,19 confirming the universality of transgenes for reprogramming. Species such as the pig, whose body and organ size, anatomy, longevity, and physiology resemble the human more than the mouse, are important in transplantation biology. Porcine iPSCs potentially fill the gap between the transplantation of these cells into mice and the initiation of the first clinical trials using iPSCs in humans.

For iPSC to have utility, these inserted transgenes must be either deleted or effectively silenced after the cells have been reprogrammed. Alternatively, reprogramming must be achieved through the use of nonintegrating vectors.20,21 Further optimization and modification of differentiation protocols are needed for efficiently generating large quantities of iPSC derivatives such as endothelial cells or cardiomyocytes. Moreover, the most obvious question to be answered by preclinical studies is which type of stem cell or progenitor cell is the best candidate for therapy.7 Because persistent ischemia also limits cell survival, revascularization and improving angiogenesis could be essential components of cell-based therapy.

Finally, the findings of Gu et al point to an elephant in the middle of the cardiac cell therapy room. Even studies that do not reveal long-term myocardial engraftment of transplanted cells generally reveal at least a temporary improvement in contractile function. Is this through new myocyte generation, protection of preexisting myocytes, or another mechanism? If there is a paracrine proangiogenic benefit of cardiac cell therapy, how does a more extensive or mature vasculature lead to contractile improvement? The mechanisms linking angiogenesis to contractile function are probably crucial to understanding the potential of cardiac cell therapy, and we simply do not understand these mechanisms sufficiently.

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None.

**References**


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