Congestive heart failure (CHF) is the only cardiovascular disease that is increasing in prevalence. Patients with CHF who have symptoms with mild activity or at rest (Class III and Class IV) have a poor long-term outcome, with 5-year age-adjusted mortality of 50%. Heart transplantation has an 80% 5-year survival rate, but only 3000 cardiac transplants are performed in the US each year, where the prevalence for severe CHF is estimated at 5 million patients. Angiotensin converting enzyme inhibitors, aldosterone antagonists, β-adrenergic receptor antagonists, and implanted cardiac defibrillators have improved survival. However, even with optimal medical and device management, heart failure is an inexorable disease associated with unacceptable high morbidity and mortality. Because the prevalence of CHF is high and the outlook dismal, new therapeutic approaches are needed.

In gene transfer experiments, one generally studies how increased expression of a single molecule influences signal transduction and cardiac function. These studies have identified a large number of potential therapeutic targets in cardiac myocytes, including cell membrane receptors and their ligands, calcium handling proteins, intracellular signaling molecules, transcription factors, and contractile proteins in cardiac myocytes. An abundance of therapeutic candidates is thwarted by the Achilles’ heel of cardiovascular gene therapy—the paucity of evidence for a vector and delivery method that will provide adequate cardiac expression safely. The exuberance engendered by cardiovascular gene therapy has subsided over the last 10 years, supplanted by similar hopes for cell-based therapy. Bone marrow–derived cells, endothelial progenitor cells, and embryonic or mesenchymal stem cells have been used successfully in animal models, although debate continues regarding cardiac myocyte regeneration per se. Clinical trials using bone marrow–derived cells in patients with acute myocardial infarction and CHF have been somewhat encouraging, but the mechanism for functional improvement may be attributable to angiogenesis rather than generation of cardiac myocytes. Thus, both gene transfer and cell-based treatments, although intellectually attractive and abounding with suitable therapeutic candidates, are hindered by challenging methodological problems.

With this backdrop, Bian and colleagues, in this issue of Circulation Research describe a novel means to treat regional dysfunction associated with myocardial infarction in rats using GATA4 gene transfer. Although GATA4 is an essential transcription factor for cardiac myocyte hypertrophy, its inability to enter cells has impeded its use in cardiovascular gene therapy. Bian and colleagues resolve this problem by engineering cultured cardiac fibroblasts to express a GATA4:VP22 fusion protein. VP22, a cell-penetrating peptide, enables GATA4 to enter cells after secretion from implanted engineered cardiac fibroblasts, thereby exerting biological effects in the targeted area, the most impressive of which is cardiac myocyte hypertrophy. In essence, Bian et al have combined cell-based therapy and gene transfer while side-stepping the baggage associated with virus vectors and stem cells.

Cell-penetrating peptides (CPP), also known as protein transduction domains, are basic and amphipathic peptides that transport proteins, peptides, plasmid DNA, siRNA, and liposomes across mammalian cell plasma membranes. The discovery of CPPs originated from the finding that the Tat transactivator of HIV virus type 1 translocates across cell membranes, its inability to enter cells has impeded its use in cardiovascular gene therapy. Bian and colleagues resolve this problem by engineering cultured cardiac fibroblasts to express a GATA4:VP22 fusion protein. VP22, a cell-penetrating peptide, enables GATA4 to enter cells after secretion from implanted engineered cardiac fibroblasts, thereby exerting biological effects in the targeted area, the most impressive of which is cardiac myocyte hypertrophy. In essence, Bian et al have combined cell-based therapy and gene transfer while side-stepping the baggage associated with virus vectors and stem cells.

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Bian and colleagues tested whether delivery of the transcription factor GATA4, in the form of a GATA4:VP22 fusion protein, could reduce regional cardiac dysfunction associated with myocardial infarction in rats (Figure). To ensure long term expression, the authors used liposomal transfection of an expression vector encoding GATA4:VP22 into cultured rat cardiac fibroblasts. Four weeks after coronary artery ligation, GATA4:VP22 expressing fibroblasts were injected into the infarct border region. Improved cardiac function was found both 4 and 6 weeks after cell injection. This study is important because it tested the potential therapeutic effects of GATA4, an essential transcription factor for cardiac hypertrophy, using cell-based gene transfer in a clinically relevant animal model of cardiac dysfunction.
Green fluorescent protein fused with VP22 exhibits paracrine function—secretion from the initially transfected cell subsequently enters other cells.\(^{22}\) Using a cell culture system, Bian and colleagues established that GATA4:VP22 fusion protein translocated from cardiac fibroblasts to mesenchymal stem cells, which resulted in expression of GATA4-responsive genes. Myocardial injection of fibroblasts expressing GATA4:VP22 was associated with immunodetectable GATA4:VP22 in cardiac myocytes. Protein expression of 2 GATA4-responsive genes, namely, cardiac myosin heavy chain and Bcl-2, were increased in GATA4:VP22 positive cardiac myocytes. These findings suggest that GATA4:VP22 translocates from fibroblast to cardiac myocyte and regulates gene expression.

Echocardiography showed increased fractional shortening 4 weeks \((P=0.013)\) and 6 weeks \((P=0.036)\) after cell transplantation. This was associated with improved radial strain in the viable region, increased cardiac myocyte cross-sectional dimension in the infarct border region, and decreased collagen deposition. This strategy, which combines gene transfer and cell transplantation, appears to hold promise for possible clinical application.

To advance this conceptually attractive approach, several areas will require further consideration. For example: (1) Efficiency of intercellular trafficking. Some reports have failed to show detectable VP22 fusion proteins after delivery to target cells,\(^{28,29}\) which underscores the technical challenges associated with this approach. Further studies focused on which individual CPPs translocate across the cell membrane most efficiently,\(^{30}\) which likely will be cell-specific, will promote optimal efficacy. Developing small molecules which mimic CPP function may also help to increase trafficking efficiency.\(^{31}\) (2) Targeting to specific cells. Resident cardiac fibroblasts contribute to the development and progression of CHF.\(^{32}\) Targeting potential therapeutic proteins specifically to cardiac myocytes or cardiac fibroblasts is likely to have advantages in efficacy and in uncovering mechanisms for beneficial effects. (3) CPP fusion protein delivery. Bian and colleagues injected GATA4:VP22 expressing cardiac fibroblasts into 5 sites of the infarct border region,\(^{16}\) which presumably led to an enduring but heterogeneous supply of GATA4:VP22 \((<1 \text{ mm}^2 \text{ range})\) and a corresponding variation in cardiac myocyte size. Will this heterogeneity lead to cardiac arrhythmias? Can the delivery method be refined so that thoracotomy and direct intramuscular delivery are not required? The authors used cultured cardiac fibroblasts as a vector for the therapeutic transgene. In potential clinical applications, it will be important to determine whether autologous fibroblasts from a source other than the patient’s heart can be used. This would be a prerequisite for translation of this method to clinical settings. Alternatively, adeno-associated virus vectors encoding GATA4:VP22 driven by an ischemia-responsive promoter could be used. Indeed, transgene expression, whether cell-based or vector-based, may require regulation because unbridled GATA4 expression may be deleterious.\(^{25}\) (4) Physiological effects. Bian and colleagues measured fractional shortening by echocardiography. Fractional shortening, an ejection phase index of cardiac function, is not an optimal measure to assess contractile function in infarcted hearts. Studies that directly assess global and regional contractility would provide additional evidence for a beneficial physiological effect. Mechanistic studies on how increased GATA4 expression and activation\(^{33}\) affect cardiac function are also needed (the relative importance of apoptosis versus hypertrophy, for example).

Despite these limitations, their studies are both novel and exciting. Bian et al have shown that transcription factors, previously difficult to use as therapeutic transgenes, can be effective when expressed as fusion proteins with CPPs. That the approach avoids the use of retrovirus vectors for engineering cells is an added advantage, given the recent reports of deleterious effects associated with such approaches.\(^{34}\) Bian and colleagues should be congratulated for their compelling study showing the beneficial effects of a GATA4 CPP fusion protein gene transfer on regional dysfunction associated with myocardial infarction.
Sources of Funding
This work was supported by National Institutes of Health grants 5P01HL066941 and HL081741 and a Merit Review Award from the Department of Veterans Affairs.

Disclosures
None.

References
Cell-Based GATA4 Cardiac Gene Transfer Using Cell-Penetrating Peptide
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Circ Res. 2007;100:1540-1542
doi: 10.1161/CIRCRESAHA.107.101102
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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