

The Promise of Cardiac Regeneration by In Situ Lineage Conversion

Although remarkable advances in acute coronary care have significantly improved outcomes following myocardial infarction (MI), survivors often experience progressive heart failure, a devastating condition with limited curative options. Given that the adult heart cannot regenerate heart muscle cells to restore contractile function, there is great demand to develop therapeutic strategies to build new heart muscle (Figure). Thus, many strategies have been conceived to induce de novo generation of cardiomyocytes for heart repair.

By far the most well-studied approach over the past 2 decades is transplantation of various cell types (bone marrow-, adipose tissue-, peripheral blood-, and cardiosphere-derived cells; skeletal myoblasts; and differentiated pluripotent stem cells) into the injured heart via multiple delivery routes (ie, intramyocardial, intracoronary, and peripheral injection). However, an alternative strategy of directly switching 1 cell type into another without transitioning through a pluripotent state (referred to as direct reprogramming) has recently emerged.

Following the identification of 4 transcription factors (TFs) that can generate induced pluripotent stem cells, it has become widely accepted that ectopic expression of cell type-specific TFs in fibroblasts can establish any new cellular identity, provided that the right factor or combination of factors is known. A core set of evolutionarily conserved TFs (ie, Gata4, Hand, Isl1, Mef2, MesP1, Nkx2-5, and Tbx5) controls cardiac gene expression and heart development. These factors bind conserved *cis*-regulatory sequences in the control regions of cardiac genes and also cross-regulate each other's expression to establish feed-forward regulatory loops that propel and sustain the cardiac developmental gene program. Thus, it is intuitive that ectopic overexpression of these factors can convert nonmyocytes toward a cardiac fate. Srivastava's group first proved this concept by demonstrating that the combination of 3 cardiogenic TFs including Gata4, Mef2c, and Tbx5 (GMT) converts mouse fibroblasts into induced cardiomyocytes (iCMs).¹

Building on this discovery, multiple investigators focused on 2 major challenges to clinical translation of this exciting new technology: (1) *in vivo* cardiac reprogramming and (2) human cardiac reprogramming. Direct intramyocardial delivery of a viral cocktail consisting of GMT or Gata4, Mef2c, Tbx5, and Hand2 (GHMT) in a mouse MI model induced iCMs from fibroblasts, leading to significant improvement in cardiac contractile function and reduction of scar formation.^{2,3} Furthermore, various TF combinations are capable of converting human fibroblasts into contractile iCMs.

Conversion of fibroblasts into cardiomyocytes is a particularly attractive strategy for treating myocardial injury. Fibroblasts are one of the most abundant cell types in the heart and become activated following an MI, culminating in fibrosis, scar formation, and adverse remodeling. Thus, targeting of activated cardiac fibroblasts following injury offers several advantages. First, direct conversion of

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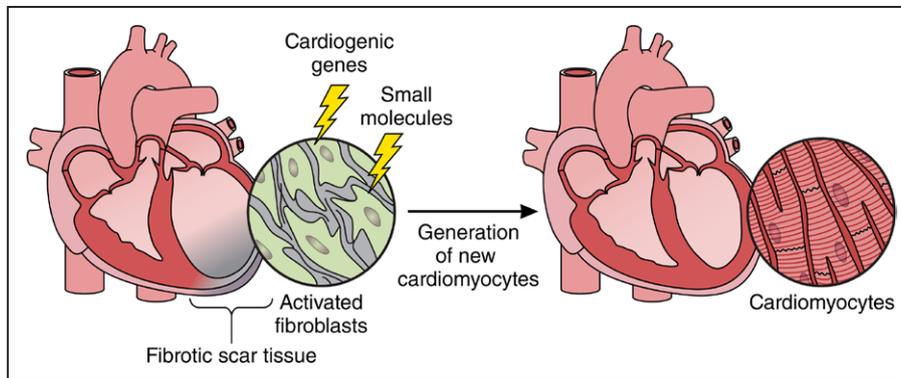


Figure. Generation of new cardiomyocytes by direct reprogramming of cardiac fibroblasts in situ.

Following a myocardial infarction (MI), a fundamental issue remains the irreversible loss of cardiomyocytes with subsequent replacement by fibroblast-derived scar tissue (**left**). Although current pharmacological treatments can slow the progression of heart failure, the ultimate therapeutic goal would be to completely restore normal cardiac function (**right**). Recent work has shown that activated fibroblasts in the MI border zone can be directly converted into functional cardiomyocytes by the addition of transcription factors (cardiogenic genes). In work described in this issue of *Circulation*, Mohamed et al⁴ show that specific small molecules can further augment the cardiac reprogramming process in situ.

nonmyocytes into iCMs can be accomplished in situ, which obviates the need to isolate/differentiate cells ex vivo. Second, direct reprogramming does not pass through a stem cell-like state, thus lessening the likelihood of teratoma formation. Third, conversion of fibroblasts into cardiomyocytes reduces fibrosis and scar formation by decreasing the pool of activated fibroblasts after an MI, thus preventing the adverse remodeling that contributes to contractile dysfunction and arrhythmogenesis.

Despite significant progress in direct cardiomyocyte reprogramming over the past several years, reprogramming inefficiency remains a significant hurdle. In contrast to induced pluripotent stem cell reprogramming, this issue is particularly critical for direct cardiomyocyte reprogramming, because reprogrammed cardiomyocytes (iCMs) do not proliferate. To repair the billions of cardiomyocytes lost during an MI, clinically significant conversion of nonmyocytes to iCMs is necessary. Toward this goal, Srivastava's group recently made an important step, described in this issue of *Circulation*, by combining a chemical approach with conventional genetic reprogramming.⁴ Through high-throughput chemical screening, they identified 2 classes of small molecules, Tgf- β and Wnt inhibitors, that significantly enhance reprogramming of postnatal mouse cardiac fibroblasts to iCMs by up to 8-fold. In addition, the functionality of iCMs was substantially improved by adding the 2 selected compounds during reprogramming.

Many previous studies have optimized the cardiomyocyte reprogramming protocol in vitro using mouse fibroblasts, but Srivastava's group invested a great deal of effort toward clinical translation of direct cardiomyocyte reprogramming. In addition to evaluating reprogramming efficiency and quality in mouse fibroblasts in vitro, they tested the efficacy of the selected

drugs on in vivo cardiomyocyte reprogramming in the context of MI in a mouse model and on in vitro human cardiomyocyte reprogramming. Combining Tgf- β and Wnt inhibitors with the introduction of a GMT retroviral cocktail post-MI significantly reduced scar formation and improved ejection fraction as measured by cardiac MRI. In addition, the new chemical protocol enhanced the efficiency and quality of human fibroblast reprogramming and made dispensable 3 of the 7 genetic factors previously shown to be necessary for human cardiac reprogramming.

The next major challenge for future clinical translation of direct cardiomyocyte reprogramming will be to develop a virus-free reprogramming protocol that eliminates the potential for genomic integration and subsequent tumorigenesis. The recently described chemical approach to directly reprogram fibroblasts into iCMs still requires at least 3 or 4 genetic factors for mouse or human reprogramming, respectively. However, the same group has shown that an all-chemical approach can induce cardiomyocyte-like cells from human fibroblasts, although this method requires transitioning through a cardiac progenitor intermediate⁵ rather than direct lineage conversion. Nevertheless, this study suggests that chemically mediated human cardiac reprogramming might be just beyond the horizon. Collectively, these studies provide hope that we may eventually be able to achieve direct cardiomyocyte reprogramming without the need for genetic delivery of the reprogramming factors, an exciting future prospect for cardiac regenerative medicine.

DISCLOSURES

None.

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FOOTNOTES

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