Induction of Cardiomyocytes From Cardiac Fibroblasts

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Study Hypothesis

The laboratory of which the authors are part had previously reported that a combination of 3 genes encoding transcription factors (Gata4, Mef2c, and Tbx5) could reprogram various types of fibroblasts into cells that had cardiomyocyte-like properties in vitro and that could be successfully transplanted into mouse heart; the authors hypothesized that delivery of these 3 genes directly into mouse heart following myocardial infarction could reprogram proliferating cardiac fibroblasts into functional cardiomyocytes in situ and thereby improve cardiac function. This is of particular interest because cardiac fibroblasts represent a large proportion of the cells in the heart and thus would serve as a ready pool for the generation of new muscle.

How Was the Hypothesis Tested?

The authors used a retroviral system to deliver the 3 reprogramming factors (Gata4, Mef2c, and Tbx5), as well as a gene encoding a red fluorescent marker into cells in mouse hearts in situ via direct intramyocardial injection. To assess the reprogramming effect of the factors in the setting of myocardial infarction, they induced myocardial injury by coronary artery ligation, followed by injection of the factors at the border of the infarct zone. They assessed the growth of cardiomyocyte-like cells in the injected areas by identifying cells that were positive for the injected red fluorescent marker gene, as well as positive for immunofluorescent staining for cardiac-specific proteins. In order to determine whether any new cardiomyocyte-like cells that were generated were truly the products of reprogramming of cardiac fibroblasts or whether they were the result of proliferation of preexisting cardiomyocytes or fusion of cardiomyocytes with fibroblasts (or other cell types), the authors used a lineage tracing approach, whereby they were able to genetically distinguish different cell types with permanent markers (whether intrinsically fluorescent markers, proteins that can be detected with immunofluorescent staining, or enzymes that catalyze reactions resulting in color staining); any new cardiomyocytes would display their cellular origin(s) via whatever marker(s) they expressed.

The authors then assessed whether any new cardiomyocyte-like cells had properties similar to preexisting cardiomyocytes. Such properties included the expression of proteins involved in cell-cell communication, passage of dyes of various sizes between cells, contraction in response to electric stimuli, generation of action potentials, and display of intracellular calcium transients. Finally, the authors determined whether the appearance of new cardiomyocyte-like cells correlated with improvements in myocardial function, as measured by echocardiography and magnetic resonance imaging, and reduction of infarct scar size, as well as increased incidence of arrhythmias, which has been an ongoing concern in cardiac cellular transplantation studies.

Principal Findings

The authors found that injection of the reprogramming factors after myocardial infarction resulted in the generation of a significant number of new cardiomyocyte-like cells over the next 4 weeks. Lineage tracing found that these cells had, at one time, expressed periostin and fibroblast-specific protein 1 (Fsp1) (arguing that they originated from cardiac fibroblasts) but had not expressed genes specific for cardiomyocytes or endothelial cells. The new cardiomyocyte-like cells expressed a number of genes specific for myocytes, including those encoding [alpha]-actinin, tropomyosin, and cardiac troponin T. The induced cells also displayed a number of morphological and electrophysiological characteristics compatible with ventricular cardiomyocyte identity and, furthermore, appeared to be electrically coupled to native cardiomyocytes. Relative to control animals, mice that received the reprogramming factors after myocardial infarction displayed improved left ventricular contractile function and smaller infarct zones 2 to 3 months later; there was no electrocardiographic evidence of more arrhythmias as a result of the reprogramming.

Implications

This study is noteworthy in that it provides a proof of principle that reprogramming of cardiac fibroblasts into functional cardiomyocytes can be performed in situ in a living mammal in the setting of myocardial infarction. One can envision delivery of vectors encoding the 3 reprogramming factors into...
myocardial tissue via a catheter-based approach in a patient who has suffered an acute coronary syndrome. Another potential application might be for patients with advanced chronic heart failure, although this study did not address whether cellular reprogramming may have use in that clinical scenario. Of note, a second study demonstrating successful reprogramming of cardiac fibroblasts into cardiomyocytes in situ was published at the same time as the study discussed here, further reinforcing the promise of this translational work.

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**Disclosures**

None.

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