

## Issues of the Heart – Directed Reprogramming to Mend a Broken Heart

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Cardiovascular disease remains the single leading cause of death worldwide [1]. At the mechanistic level, this is largely attributed to the limited ability of the heart to undergo regeneration following an injury such as myocardial infarction (heart attack). Dead cardiomyocytes are replaced by fibroblasts, leading to the accumulation of fibrotic scar tissue that results in impaired cardiac function, heart failure and death.

Cell-based therapies for regenerating the heart have been a major focus of research efforts in cardiac regenerative medicine. However, generating enough cardiomyocytes to repair a damaged heart has been the rate limiting step for the field's advancement. It is estimated that more than 1 billion cells are required to repair a heart following a myocardial infarction, highlighting the need for high-throughput and reproducible methodologies for *de novo* cardiomyocyte production.

With the isolation of human embryonic stem cells (ESC) by Thomson and colleagues in 1998 [2], followed more recently by reports of the ability to reprogram somatic cells to pluripotent state (induced pluripotent stem cells, iPSC) [3], research has rapidly shifted to developing strategies to efficiently and reliably direct stem cells into the cardiovascular lineage. Since the initial demonstration that contracting cardiomyocytes can be generated from human pluripotent ESC and iPSC, the possibility of producing unlimited numbers of human cardiomyocytes to rebuild the heart has tantalized researchers. However, the use of pluripotent cells confers an increased risk of tumor formation and uncontrolled lineage differentiation of the injected cells.

As an alternative source of *de novo* cardiomyocytes, efforts have focused on "direct reprogramming" of cardiac fibroblasts and other adult cell types into cardiomyocytes using cardiac-specific transcription factors. In 2010, Srivastava's group demonstrated that a combination of just three transcription factors that play a role in regulating heart development (*Gata4*, *Mef2c* and *Tbx5*) was sufficient to directly reprogram cardiac and somatic fibroblasts into cardiomyocyte-like cells *in vitro* [4]. Although these reprogrammed cardiomyocyte-like cells functioned at only partial efficiency compared to fully differentiated native adult cardiomyocytes in terms of their electrophysiological and contractile properties, this study nevertheless demonstrated the feasibility of this approach.

Most recently, two follow up papers published back-to-back in *Nature* both demonstrated that direct injection of retroviral vectors harboring combinations of cardiac-specific transcription factors to an injured heart can reprogram non-cardiomyocytic cells into the cardiac lineage and improve cardiac function in live mice. Srivastava's group expanded upon their initial *in vitro* findings and demonstrated that direct local injection of retroviral vectors harboring *Gata4*, *Mef2c* and *Tbx5* into infarcted murine hearts was sufficient to convert resident cardiac fibroblasts into cardiomyocytes, and importantly, to decrease infarct size and improve cardiac functions such as ejection fraction, stroke volume and total cardiac output [5]. The addition of thymosin- $\beta$ 4, which promotes angiogenesis, further enhanced these effects [5]. Similarly, Olsen's group also used retroviruses to deliver a cocktail of four factors (*Gata4*, *Mef2c*, *Tbx5* and *Hand2*) and reported similar findings [6]. To demonstrate that the observed effects were the result of the reprogrammed fibroblasts and not due to pre-existing cardiomyocytes, lineage tracing studies were carried out where

cardiomyocytes expressing the transcription-factor cocktail were selectively marked.

Cellular reprogramming efficiencies are notoriously low. What is perhaps most surprising from these studies is that even though only a relatively low number of cells underwent lineage reprogramming (2.4-6% in the study by the Olsen group [6], and 35% with thymosin- $\beta$ 4 addition reported by Qian et al. [5]), the global improvement to heart function was significant. This raises the possibility that in addition to contributing to regeneration, these cells may be involved in producing growth factors, cytokines or other signaling molecules that may either improve the performance of pre-existing, dormant cardiac progenitor cells or participate in the recruitment of other cells to repair the damage.

Currently, transplantation remains the last resort for patients suffering from cardiovascular disease. However, with the severe shortage of donor organs and the risk of immunological rejection following transplantation, other treatments are desperately needed. The major challenge limiting the cell regeneration approach has been generating sufficient cells to repair the damage. The recent reports by Qian et al. and Song et al. open up a new line of investigation in cardiovascular medicine and bring us a step closer to using cell-based therapies in the clinic. A logical next step would be to address the most efficient way to deliver these agents or cells to maximize engraftment efficiency and this is where multi-disciplinary collaborations between biologists, bioengineers, and clinicians are needed. Although many more questions still need to be addressed through intensive research, our current ability for cellular manipulation is developing rapidly and will ultimately lead to novel therapies to repair a broken heart.

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