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RESEARCH HIGHLIGHT

In vivo reprogramming for heart disease

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The term "lineage reprogramming" is typically used to describe the conversion of one differentiated somatic cell type into another without transit through a pluripotent intermediate. Two recent reports in *Nature* demonstrate that such a conversion can be achieved in the heart *in situ*, and suggest a novel, regenerative approach for the development of cardiac therapeutics.

Heart disease is the leading cause of morbidity and mortality worldwide [1]. The end state of degenerative heart disease is heart failure, which is associated with loss of pump function and increased susceptibility to life-threatening arrhythmias. In the field of regenerative cardiovascular medicine, the most extensively described therapeutic strategy for replacement of lost heart tissue involves transplantation of autologous somatic cells. Recent clinical studies have demonstrated that this type of cell-based therapy can be done relatively safely, but the clinical results suggest the need for improved strategies [1]. This cell-based approach is associated with many hurdles, including difficulties in producing enough cells to ensure a clinically meaningful effect, poor cell survival and differentiation of transplanted cells *in situ*. In this regard, recent reports by Qian *et al.* [2] and Song *et al.* [3] in *Nature* describe a novel, transplantation-free strategy to repair an injured heart through *in vivo* lineage reprogramming, which might circumvent several of the problems associated with transplantation of exogenous cells.

Lineage reprogramming describes the conversion of differentiated cells directly into other somatic cell types, without first "de-differentiating" the originating cells into a pluripotent intermediate population. This general concept was first clearly established by the conversion of fibroblasts to skeletal myoblasts by the forced expression of MyoD in 1987, and was followed by more examples of lineage reprogramming, including conversion of B lymphocytes to macrophages, inner ear support cells to hair cells, exocrine pancreatic cells to endocrine β-cells, and fibroblasts to neurons [4].

A lineage reprogramming strategy for conversion of fibroblasts to cardiomyocytes was first reported by Ieda *et al.* in 2010 [5]. Lineage reprogramming of fibroblasts into cardiomyocytes is a theoretically compelling solution to the clinical problem of fibroblast proliferation and scar formation after myocardial infarction. Scar formation is associated with both a decrease in cardiac pump function and an increase in the frequency of arrhythmias. Ieda *et al.* [5] demonstrated that cultured mouse fibroblasts could be converted to cardiomyocyte-like cells *in vitro* through ectopic expression of three cardiogenic transcription factors: Gata4, Mef2c and Tbx5 (GMT). However, the efficiency of such a conversion seemed extremely low. Few cells in which the aforementioned transcription factors were expressed adopted a phenotype resembling that of a cardiomyocyte. Song *et al.* [3] reported that addition of a fourth factor, Hand2, could increase the efficiency of the conversion. Even with four factors (GHMT), the conversion efficiency was low (~1%).

It was proposed that the low efficiency of *in vitro* lineage reprogramming could be due to the lack of a natural environment for cardiomyocytes [2, 3]. Based on this assumption, it might follow that factors present *in vivo* would promote the conversion toward cardiomyocytes and maintenance of the mature phenotype. In addition, *in vivo* reprogramming can avoid several problems associated with transplantation. Therefore, it would be interesting to test the feasibility of reprogramming cardiac fibroblasts to cardiomyocytes *in vivo*. Indeed, Qian *et al.* [2] and Song *et al.* [3] reported that *in vivo* lineage reprogramming of fibroblasts into cardiomyocytes was possible, and the efficiency was significantly higher (up to 12%) than *in vitro*. Both groups performed lineage reprogramming by injection of retroviral particles containing the cardiogenic transcription factor genes (GMT for Qian *et al.* or GHMT for Song *et al.*) into mouse hearts at the

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time of programmed myocardial infarction. Reprogramming events were detected about 4 weeks later. Some cardiac fibroblasts appeared to transdifferentiate into cells with a phenotype very similar to cardiomyocytes, which were either called induced cardiomyocyte-like cells (iCMs) by Qian *et al.* or induced cardiac-like myocytes (iCLMs) by Song *et al*. Lineage tracing studies using Cre drivers with enriched expression in fibroblasts indicate that the vast majority of iCMs/iCLMs originated from cardiac fibroblasts, and were created *de novo*. Cell fusion between fibroblasts and pre-existing cardiomyocytes was ruled out by the observation that the cardiomyocyte population genetically pre-labeled was actually diluted by newborn cardiomyocyte-like cells. Use of this technique to convert fibroblasts to cardiomyocytes was associated with reported reduction in scar size and increase in left ventricular ejection fraction 8-12 weeks after infarction-associated treatment.

These studies conform to the concept of lineage reprogramming; however, it has been suggested that interpretation of "reprogramming" results ought to be approached with caution [6]. Authentic reprogramming events would require the resetting of the epigenome in the parental cells, demonstrated by the adoption of the molecular and cellular features of the destination cell type, including patterns of epigenetic markers, gene expression, morphology and functionality, which should be stable on a long-term basis. Ectopic expression of some transcription factors can activate their direct or indirect downstream targets, especially when they are in a genetic network, as is the case for several transcription factors in the cardiomyocyte lineage [7]. Activation of tissue-specific genes or the gain of certain phenotypic features of the destination cells can be a reflection of partial lineage reprogramming, which can result in a spectrum of intermediate phenotypes with various degrees of similarity to the defined destination cell type, as observed by Song *et al*. [3]. Chen *et al.* [8] and Protze *et al.* [9] recently contended that the three factors (GMT) used by Ieda *et al*. [5] could activate the expression of a subset of cardiac genes in fibroblasts, but the cells were not completely converted to cardiomyocytes *in vitro*. On the other hand, Jaywawardena *et al.* [10] reported that the transient expression of a single microRNA could convert fibroblasts to cardiomyocyte-like cells. It appears that multiple combinations of transcription factors or microRNA(s) can lead fibroblasts to gain certain features of cardiomyocytes, although a more detailed understanding of the lineage reprogramming process will be required in order to distinguish between genuine cardiomyocytes and cardiomyocyte-like cells. It will become particularly important to document the relative extent of differentiation of these cardiomyocyte-like cells, as well as their maturity (fetal versus adult), stability and durability of normal function, and long-term survival.

As with any new, exciting therapeutic regenerative strategy, a number of hurdles will need to be passed before lineage reprogramming can be considered as a viable therapeutic strategy for cardiac regeneration. It will be necessary to demonstrate that expression of a small number of transcription factors can be used to convert human fibroblasts into cardiomyocytes (as Qian *et al.* and Song *et al.* demonstrated in mouse fibroblasts). One of the central challenges to any type of nucleotide-based therapy has been *in vivo* delivery systems, particularly those that can achieve a level of efficiency of gene delivery in an interventional (catheter-based) versus surgical approach. In the studies published by Qian *et al.* and Song *et al.*, retroviruses were used to introduce the transgenes into the infarction area. The retroviral integration in general has been shown to increase the incidence of cancer, as demonstrated in several cases of retrovirus-based gene therapy [11]. It will be necessary to find a safe, non-integrative method to induce the reprogramming *in vivo*. Once a safer delivery agent is identified, its efficiency will need to be assessed.

While a reduction in scar burden may improve pump function, one of the central problems in heart failure is the onset of life-threatening ventricular arrhythmias that can arise from electrical heterogeneity of the cardiomyocytes within the intact myocardium. It is generally accepted that the substrate of arrhythmias associated with ischemic cardiomyopathy is based on zones of slow conduction that are comprised of viable cardiomyocytes interdigitated with fibroblasts and connective tissue [12]. Since it is likely that there will be topographically heterogeneous lineage distribution arising from the *in vivo* reprogramming driven by the 3-4 genes, it will be important to examine whether there is an increased risk of arrhythmias in larger animal model systems where this can be thoroughly examined.

Nevertheless, the *in vivo* reprogramming techniques described by Qian *et al.* and Song *et al.* may be the groundwork of a novel strategy to treat degenerative heart disease. The disparity between the *in vitro* and *in vivo* efficiency points to critical paracrine factors that may be required for this process, and that may have effects comparable to the combinatorial gene cocktail itself. This approach joins an exciting list of novel therapeutic approaches being developed to attempt to drive heart regeneration, including autologous and allogeneic non-cardiac cell-based therapy [1], expansions of rare endogenous heart cells for autologous therapy [1], design of heart patches from pluripotent stem cells [13], and the transplantation of heart progenitors and/or their differentiated cell types from pluripotent cell lines [14, 15]. A convergence of the fields of cardiac developmental biology, heart stem cell biology, tissue engineering, interventional device delivery technology, and

cardiovascular clinical medicine is on the horizon. Given the complexity of this goal and the diversity of technology required, it is likely that interdisciplinary teams will be required, i.e., an Apollo mission for heart regenerative therapeutics. For heart failure patients worldwide, this would clearly be "one small step for man, and a giant leap for mankind".

References

1 Ptaszek LM, Mansour M, Ruskin JN, Chien KR. Towards regenerative therapy for cardiac disease. *Lancet* 2012; **379**:933-942.

2 Qian L, Spencer CI, C Ian Spencer, *et al*. *In vivo* reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* 2012; **485**:593-598.

3 Song K, Luo X, Qi XQ, *et al*. Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature* 2012; **485**:599-604.

4 Asuelime GE, Shi Y. A case of cellular alchemy: lineage reprogramming and its potential in regenerative medicine. *J Mol Cell Biol* 2012; **4**:190-196.

5 Ieda M, Fu JD, Delgado-Olguin P, *et al.* Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 2010; **142**:375-386.

6 Zhou Q, Melton DA. Extreme makeover: converting one cell into another. *Cell Stem Cell* 2008; **3**:382-388.

7 Olson EN. Gene regulatory networks in the evolution and development of the heart. *Science* 2006; **313**:1922-1927.

8 Chen JX, Krane M, Deutsch MA, *et al.* Inefficient reprogramming of fibroblasts into cardiomyocytes using Gata4, Mef2c, and Tbx5. *Circ Res* 2012; **111**:50-55.

9 Protze S, Khattak S, Poulet C, *et al.* A new approach to transcription factor screening for reprogramming of fibroblasts to cardiomyocyte-like cells. *J Mol Cell Cardiol* 2012; **53**:323-332.

10 Jayawardena TM, Egemnazarov B, Finch EA, *et al.* MicroRNA-mediated *in vitro* and *in vivo* direct reprogramming of cardiac fibroblasts to cardiomyocytes. *Circ Res* 2012; **110**:1465-1473.

11 Modlich U, Baum C. Preventing and exploiting the oncogenic potential of integrating gene vectors. *J Clin Invest* 2009; **119**:755-758.

12 Donahue JK. Gene therapy for ventricular tachyarrhythmias. *Gene Ther* 2012; **19**:600-605.

13 Domian IJ, Chiravuri M, van der Meer P, *et al.* Generation of functional ventricular heart muscle from mouse ventricular progenitor cells. *Science* 2009; **326**:426-429.

14 Laflamme MA, Chen KY, Naumova AV, *et al.* Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 2007; **25**:1015-1024.

15 van Laake LW, Passier R, den Ouden K, *et al.* Improvement of mouse cardiac function by hESC-derived cardiomyocytes correlates with vascularity but not graft size. *Stem Cell Res* 2009; **3**:106-112.