

Reprogrammed Cardiac Fibroblasts to the Rescue of Heart Failure

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In Vivo Reprogramming of Murine Cardiac Fibroblasts Into Induced Cardiomyocytes

Qian et al
Nature. 2012;485:593–598.

Heart Repair by Reprogramming Nonmyocytes With Cardiac Transcription Factors

Song et al
Nature. 2012;485:599–604.

Heat failure is a growing problem, but 2 recent reports in *Nature* provide new insights into the role of reprogramming cardiac fibroblasts in damaged mouse hearts into cardiac myocytes. The enhanced benefits of cardiac fibroblast conversion into cardiomyocytes may provide new therapeutic venues for the treatment of heart failure.

Heart failure is a leading cause of death worldwide. It is often the result of acute or chronic ischemic heart injury. Because the adult heart does not have the capacity to replace the injured cardiomyocytes, this inevitably leads to loss of cardiomyocytes, fibrosis, and loss of pump function. Many efforts have been put into cell-based therapy, in hope that exogenously delivered cells could replace injured cardiomyocytes and restore pump function. However, efforts up to date only led to a limited degree of success. In a recent issue of *Nature*, Qian et al from the Srivastava laboratory and Song et al from the Olson laboratory have made a major breakthrough in the research for the treatment of heart failure.^{1,2} The two groups have independently shown that cardiac fibroblasts can be reprogrammed into cardiomyocyte-like cells in vivo, accompanied by pronounced improvement of cardiac contractile function after myocardial infarction.

In 1987, Davis et al³ first demonstrated that a single transcription factor, MyoD1, can convert fibroblasts into myoblasts. The search for a “CardioD” has been underway for many years, but only Mesp1 has recently been reported as the likely candidate.^{4,5} Shinya Yamanaka’s discovery of induced pluripotent stem cells (iPSCs) has injected new hope to this line of research: patients’ own cells can be reprogrammed into a stem cell fate and then

directed to the desired cell type for therapy.⁶ Inspired by Yamanaka’s work, others have proven that adult somatic cells can be directly reprogrammed into other cell types, including cardiomyocytes.

The work of Qian et al is based on a previous publication of the same laboratory, in which Gata4, Mef2c, and Tbx5 (GMT) were screened out of 14 transcription factors to reprogram cardiac or tail-tip fibroblast into cardiomyocyte-like cells in vitro.⁷ In the current study, Qian et al used 2 genetic approaches (periostin-Cre/Rosa26R-LacZ and fibroblast-specific protein 1-Cre/Rosa26R-LacZ) to mark noncardiomyocytes (mostly cardiac fibroblasts) in vivo, performed ligation of the left anterior descending artery, then delivered a high concentration of a mix of GMT retrovirus into the infarct/border zone. At 4 weeks after myocardial infarction, 35% of cardiomyocytes from the infarct/border zone were of noncardiomyocyte origin, as evidenced by double positive staining of β -galactosidase and α -actinin. At 8 and 12 weeks after myocardial infarction, MRI and echocardiography studies showed marked improvement of pump function in the GMT-treated group over controls.¹

Song et al found that an extra transcription factor, Hand2, in addition to GMT in their reprogramming cocktail (GHMT), was more efficient than the GMT recipe in reprogramming cardiac fibroblasts, isolated from an α -MHC-GFP reporter mouse line, toward cardiomyocyte-like cells. Song et al also used 2 genetic approaches to mark the noncardiomyocyte populations for in vivo reprogramming of cardiac fibroblasts. One approach used fibroblast-specific protein 1-Cre/Rosa26R-LacZ, as was used by Qian et al.⁵ The second approach used mice carrying Tcf21-iCre/Rosa26R-tdTomato genes, which required administration of tamoxifen to induce Cre expression and mark the noncardiomyocyte population before left anterior descending artery ligation. The viral cocktail of the 4 GHMT factors injected into hearts resulted in fibroblast-originated cardiomyocytes (2.4% to 6.5%) in the infarct/border zone. Despite a relatively small presence of reprogrammed cells, the GHMT-treated group showed marked improvement of pump function and decrease of scar size.²

Although none of the tracing strategies are exclusive to cardiac fibroblast, collectively the evidence has reasonably established that the reprogrammed cardiomyocyte-like cells are of cardiac fibroblast origin. Furthermore, the programmed cells have acquired characteristics such as spontaneous contraction, cardiac electric potential and sarcomere structure, attesting to their close resemblance to cardiomyocytes. Before we can move to the next stage of using this new technology for heart failure therapy, however, there are a few questions to be addressed down the road.

The mechanism of the improvement of cardiac function is still not clear. To almost all cell-based therapy, it is difficult to distinguish if the effect is due to the cell replacement/

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Commentaries serve as a forum in which experts highlight and discuss articles (published elsewhere) that the editors of *Circulation Research* feel are of particular significance to cardiovascular medicine.

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(*Circ Res*. 2012;111:831-832.)

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Circulation Research is available at <http://circres.ahajournals.org>
DOI: 10.1161/CIRCRESAHA.112.279745

reprogramming per se or the change of the microenvironment toward favorable for existing repairing mechanisms. Thus, the reprogramming process could have promoted the release of growth factors, inflammatory factors that led to vasculogenesis and better cardiomyocyte survival after myocardial infarction, which could not be ruled out. A specific question toward targeting cardiac fibroblasts is whether redirecting them away from scar formation is sufficient for function improvement. Indeed, the percentages of reprogrammed cells in the infarct/border zone were drastically different between the 2 studies, yet the improvements of pump function were comparable.

The selection of transcriptions factors may be further refined. Of the 3 studies published thus far, including the aforementioned 2 studies, the screening outcome was somewhat different.^{2,7,8} Song et al found that Hand2 was beneficial for optimized reprogramming, whereas the earlier study from the Srivastava laboratory did not find this.^{2,7} A third study from Protze et al⁸ screened 120 possible triplet combinations of 10 transcription factors and found that Myocardin, Mef2c, and Tbx5 were most potent in reprogramming mouse embryonic fibroblasts, and the addition of Gata4 was detrimental. These discrepancies suggest that the cell type used, the vector carrying the transcription factor, the culture condition, and the reporter system could all affect the outcome of screening. We should be especially cautious about the reporter systems. Because cardiac transcription factors often reside on the promoter of cardiac structure genes, it is predictable that screening with cardiac structure gene–based reporter systems may lead to isolated activation events, for example, the activation of a structure gene–based reporter but not the full cardiac program. Therefore, screening with structure gene–based reporters should only serve as the first step, and isolated events in such screenings should be avoided. Other indices for testing reprogramming such as the use of functional reporters, for example, cardiac electric potential, should also be used.

The optimal destination cell type to reprogram into remains to be determined. Though the cardiomyocyte is an obvious choice for the purpose of repairing a failing heart, cardiac progenitors represent a legitimate alternative because they offer the advantage of multipotency and higher proliferation. For this notion, we have reprogrammed human dermal fibroblast into cardiac progenitor cells, with ETS2 and MESP1, factors proven to be critical for the development of *ciona intastalis* heart.⁵ Our study showed that only 2 factors, ETS2 and MESP1, were needed to reprogram human foreskin dermal fibroblasts into cardiac progenitors within a few days in tissue culture. Neither factor by itself was capable of converting fibroblasts into cardiac cells. These progenitors go on to express the 4 to 5 genes that Olson's and Srivastava's labs showed to be critical for repairing damaged mouse hearts. Besides being on top of the regulatory hierarchy, ETS2 and MESP1 converted human skin cells into intermediate staged myocytes, as one would find in early-staged embryonic hearts. Our study also used ETS2 and MESP1 proteins that can enter fibroblasts to convert noncardiogenic cells into myocytes, thus avoiding viral infections. In totality, long-term expression of reprogramming factors is not

required for maintaining the reprogrammed state, as shown by these studies.

Finally, reprogramming through microRNAs holds a unique advantage because they can be delivered systematically or locally, without a viral carrier system. Jayawardena et al⁹ have shown that miR-1, 133, 208, and 499 were effective in reprogramming fibroblasts into cardiomyocytes after myocardial infarction. Because of the lack of detailed information such as the efficiency of in vivo reprogramming and quantitative measurement of functional improvement, comparing miRNA-based reprogramming with transcription factor–based reprogramming is currently not possible.

In summary, Chen et al and Song et al have demonstrated that the resident cardiac fibroblasts, representing more than 50% of the heart cell population, can be reprogrammed to take a cardiomyocyte-like fate. Such an approach skips many obstacles that current experimental designs must face and has the added advantage of reducing scar formation and fibrosis. These studies, together with the questions and new ideas inspired by them, would probably lead us toward a new heart repair opportunity. The value of these studies, therefore, can hardly be overemphasized.

Sources of Funding

Y.L. and R.J.S. are supported by the Texas Heart Institute, with funds from the Cullen Foundation; an Advanced Research Program grant from the Texas Higher Education Coordinating Board; research funds and Cullen Distinguished Professorship of Biology and Biochemistry from the University of Houston (to R.J.S.); National Institutes of Health grants (to R.J.S.); and an American Heart Association grant (to Y.L.).

Disclosures

None.

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