

A Reliable Recipe for Heart Cells?

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Deepak Srivastava's group at the Gladstone Institute of Cardiovascular Disease, University of California San Francisco, reported in the August 6 issue of the journal *Cell* that to make heart muscle cells, all you need are three factors and some fibroblasts. But is it really that easy?

When a heart is injured, fibroblasts become activated, they proliferate, and they quickly mend the damage. But the fibrotic scar that the cells form does not contract like the muscle it replaced. The reduced global contractility means the heart has to work much harder, and the extra stress can ultimately lead to heart failure and death.

A major objective for cardiologists is to replace the lost myocytes and return functionality to the heart. One way to do this would be to introduce stem or progenitor cells to the injury site. Thus, using injured mouse hearts, scientists have tested the reparative qualities of embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, adult mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and cardiac progenitor cells (CPCs) with varying degrees of success.

With any stem or progenitor cell source, however, there are issues that need to be resolved before effective clinical use. These include the efficiency of the cells to differentiate into cardiac myocytes, the ability of the cells to engraft and function at the injury site, and the risk of tumor formation from cells that continue to grow without differentiating properly.

This summer, Deepak Srivastava and colleagues described a new technique for making cardiac myocytes that could avoid the use of stem or progenitor cells all together.¹ They reported that by transferring three transcription factors—Gata4, Mef2c, and Tbx5—into fibroblasts, they could directly transdifferentiate these cells into cardiac myocytes. “That’s extraordinarily hot,” says Robert Schwartz, Cullen Distinguished Professor of Biology and Biochemistry, University of Houston, Tex, “because you bypass the whole issue of cancer.”

Similar transdifferentiation approaches have been described recently for making β -cells directly from pancreatic exocrine cells² and for making neurons directly from fibroblasts.³ These studies follow Shinya Yamanaka’s seminal discovery that iPS cells could be made from adult fibroblasts by transfecting them with four stem cell transcription factors.⁴ It has been known for many years that numerous cell types could be reprogrammed into skeletal- and smooth muscle-like

cells through the expression of single transcription factors,^{5,6} but the recipe for activation of the cardiac muscle program had remained elusive.

If Srivastava’s approach of inducing cardiac myocytes could be used clinically, not only might it minimize cancer risk, but also it might eliminate engraftment problems too, because instead of introducing new cells, cardiologists could potentially change the fibroblasts of the scar itself.

On face value, Srivastava’s results sound very exciting. The story received a good deal of press coverage, and scientists have described the report as “a wonderful study,” “impressive,” and “fascinating.” However, for some of those scientists, the praise comes with a dose of common sense, caution, and even a hint of disbelief.

“I think that it’s potentially a significant advance. What remains to be determined is how efficient this process is, how straightforward it is to replicate, and whether it requires specialized conditions,” says Eric Olson, Professor and Chairman of Molecular Biology, University of Texas Southwestern, Dallas, Tex. “It is so important and so provocative that it is absolutely essential that it be reproduced. And not just reproduced, but reproduced easily,” says Ken Chien, the Sanders Professor in the Department of Stem Cell and Regenerative Biology at Harvard University, Boston, Mass. Summing up what seems to be the general mood among scientists in the field, Schwartz adds, “It’s an astounding observation, and many of us researchers would love to test it and see if this really works.”

A number of groups have been attempting to replicate the results of the article but have had little success, yet. But Srivastava is not worried: “I think it is healthy for the field to have skepticism about any such result that is somewhat transformative,” he says, “but time will show.”

He is sending his reagents to researchers who have asked for them, and now, it is a matter of waiting, he says. “They’ll get it to work because we’ve had many people in my lab who can make it work. . . the key to it is getting high enough levels of expression of the reprogramming factors. You need to have high-titer vectors that will infect the cells.”

Assuming Srivastava is right, that robust replication of the procedure is possible, there will be many steps to optimize and modify before there is any hope of using such a technique in the clinic. One issue is the efficiency of the transdifferentiation, says Olson. Srivastava and colleagues report that following transfection with the three factors, 20% of fibroblasts were induced to express α MHC—a marker of cardiac myocytes—but only 30% of that 20% went on to express cardiac troponin—a marker of the sarcomere structure of cardiac myocytes—and only 30% of that 30% exhibited spontaneous contraction. That works out to just 1.8% of the starting cell population that achieved the intended phenotype.

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*News & Views are edited by Aruni Bhatnagar, Ali J. Marian, and Houman Ashrafian.

(*Circ Res.* 2010;107:1042-1043.)

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Circulation Research is available at <http://circres.ahajournals.org>
DOI: 10.1161/RES.0b013e3181ffeac0

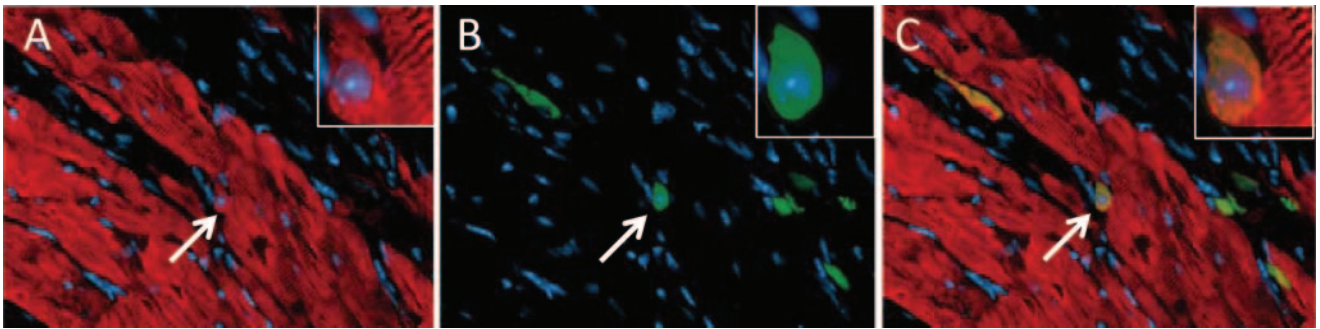


Figure. Detection of Gata4/Mef2c/Tbx5-transduced cardiac fibroblasts transplanted into a mouse heart. Panel A shows a thin myocardial section stained with an antibody against α -actinin, a marker of cardiac myocyte. Panel B shows expression of GFP in the same section, and panel C is an overlay of panels A and B. Arrow points to a cell that coexpress GFP and α -actinin. Inserts show a higher magnification of this cell. (Reprinted from Ieda et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factor. *Cell*. 2010;142:375–386. Copyright 2010, with permission from Elsevier.)

Another question is the maturity of the induced cells. Although this 1.8% of cells displayed contractile ability, whether this ability equates to the full contractile force of an adult cardiac myocyte is unknown. Also unknown is whether the cells are capable of electrically coupling to the existing heart cells *in vivo*.

The results presented in the current article are based on transfections performed on neonatal mouse fibroblasts *in vitro*. “We have tried adult fibroblasts, as well,” says Srivastava, “and they work.” But the two big questions are, will it work in human cells, and can fibroblasts of the heart be transdifferentiated directly *in vivo*, says Shinya Yamanaka, also at the Gladstone Institute of Cardiovascular Disease. “We really want to make it happen in patients, not Petri dishes,” Yamanaka says.

As it turns out, the issue of *in vivo* transdifferentiation might be among the easier hurdles to overcome: “We have data from our own lab that suggests you can do the reprogramming *in vivo* relatively more efficiently than *in vitro*,” says Olson, “It may be because the microenvironment is more conducive to this type of reprogramming or it might be that prolonged proliferation or maintenance of fibroblasts in tissue culture may impede the reprogramming process.”

If reprogramming the cells directly *in vivo* becomes a possibility, to make the switch from experimental organism to human would likely require a different factor delivery system, such as small molecules or protein transduction, neither of which are optimized. The retrovirus vector that Srivastava and colleagues used in their study integrates into the cell’s genome and, thus, can potentially cause dangerous mutations.

“The game changer is being able to do it *in vivo*,” says Chien, but points out that even if all the issues of efficiency, functionality, and safe delivery are overcome, scientists would still have to figure out how to restrict the transdifferentiation to fibroblasts: “If you hit vascular cells, nerve cells,

or pacemaker cells and start converting them to ventricular muscle, that would be a mess.”

It is not clear whether the same three factors would be able to convert other cell types into cardiac myocytes. iPS cells can be derived from fibroblasts,⁴ liver cells, stomach cells,⁷ and blood⁸ using the same four factors. Thus, if Gata4, Mef2c, and Tbx5 do turn out to be master regulators of cardiomyogenesis, the challenge will be not only to boost their power in target fibroblasts, but also to prevent their power elsewhere.

As with any paradigm-shifting study, there are many ifs, buts, and maybes between Srivastava’s proof-of-principle article and the clinic. The first and most important if, is whether researchers can validate the findings through replication. From that point, it will hopefully just be a matter of refining the recipe.

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Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Circ Res. 2010;107:1042-1043

doi: 10.1161/RES.0b013e3181ffeac0

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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World Wide Web at:

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