

## The All-Chemical Approach A Solution for Converting Fibroblasts Into Myocytes

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### Conversion of Human Fibroblasts Into Functional Cardiomyocytes by Small Molecules

Cao et al

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**Converting cardiac fibroblasts to cardiomyocytes has been considered as a regenerative strategy for myocardial infarction and other disorders. Recently, Cao et al<sup>1</sup> defined a cocktail of 9 chemical compounds with this capability, increasing the likelihood of clinical success.**

It has taken a while, but the concept that the terminally differentiated state is immutably stable no longer dominates modern biology. In 1938, Hans Spemann contemplated a fantastical experiment in which transfer of an egg nucleus could redirect a recipient somatic cell to become pluripotent, and Gurdon<sup>2</sup> made this a reality in 1958 by converting gut epithelial cells into whole frogs. Inspired by Gurdon et al,<sup>2</sup> Takahashi and Yamanaka<sup>3</sup> developed the induced pluripotent stem cell (iPSC) technology where 4 transcription factors sufficed in generating PSCs. The last decade has witnessed an explosion in cell fate manipulations. Many somatic cell types were interconverted by the means of transcription factors and epigenetic and cellular pathway modulators. As one of the most difficult cells to regenerate, cardiomyocyte was successfully generated from fibroblasts by introducing cocktails of transcription factors.<sup>4–7</sup> In a recent article in *Science*, Cao et al<sup>1</sup> reported the successful reprogramming of functional cardiomyocytes by small molecules and growth factors.

Cao et al<sup>1</sup> devised a 3-step protocol. Human foreskin fibroblasts or human fetal lung fibroblasts were first treated with 9 compounds (9C: CHIR99021, A83-01, BIX01294, AS8351, SC1, Y27632, OAC2, SU16F, and JNJ10198409) that were the outcome of multiple rounds of focused screening. Next, the cells were incubated with a cardiac induction medium containing previously known cardiogenic factors

activin A, BMP4, VEGF, and CHIR99021. Finally, human cardiomyocyte-conditioned medium was used to aid maturation. At day 30, 6.6±0.4% cells in the culture were cardiac troponin T positive, a remarkable improvement in efficiency over transcription factor-mediated reprogramming (Figure). The chemically converted cells in many ways resembled CMs derived from human PSCs. They displayed sarcomeric structures, epigenetic signatures, transcriptomes, and electrophysiological properties comparable to early-stage cardiomyocytes.

This all-chemical approach represents a major advance for cardiac regeneration. From a cell therapy perspective, it is appealing because it circumvents inadvertent genomic modifications that can occur with genetic methods, and the chemical compounds themselves are nonimmunogenic. Other potential advantages include less variability, tighter dose control, and greater cost-effectiveness if translated clinically. From a chemical biology perspective, a deeper understanding of the protein targets of the compounds and the signals they evoke constitute a new angle on understanding cardiac differentiation and reprogramming mechanisms.

### Mechanism of Cardiac Specification

9C appeared to operate exclusively in the first step of reprogramming. Treatment led to decondensation of closed chromatin regions, which was apparent by a decrease in H3K9me3, HP1γ, H3K27me3 marks and an increase in H3K4me3 and H3K27ac marks. These changes correlated with induction of genes that mark embryonic mesoderm, including EOMES, T, MESP1, and KDR that, in the context of an embryo, are expressed before the appearance of cardiac cells. A more detailed characterization of the cells induced by 9C will answer whether the cocktail directs fibroblasts to a progenitor state, such as the transient extraembryonic endoderm (XEN)-like cells that appear during chemical reprogramming toward iPSCs.<sup>8</sup> Another important question is whether 9C narrowly specifies cardiac fate or makes cells capable of following multiple lineages, such as mesendoderm progenitors created by genetic reprogramming with MESP1.<sup>9</sup> Such breadth would make 9C broadly important for tissue regeneration.

The efficiency of reprogramming was low (≈7%), raising the question of whether 9C acts deterministically, but only a few cells in the starting population are competent to respond, versus whether directed differentiation is stochastic and carries a low probability of overall success. In either case, defining the cell types that are competent to respond will also be important for translation.

### Mechanisms of Chemical Reprogramming

All-chemical cocktails have been reported to convert somatic cell into other cell types, including PSCs, neurons,

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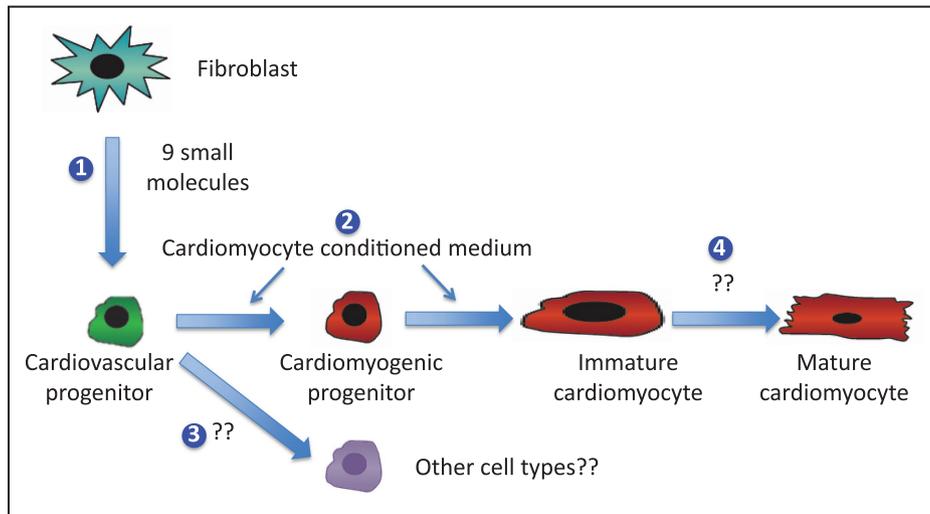
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**Figure. Cardiac reprogramming.** In a major advance, Cao et al<sup>1</sup> discovered a cocktail of 9 small molecules (1) that reprogrammed fibroblasts to cardiovascular progenitors in a process involving chromatin remodeling. Subsequent differentiation (2) to immature cardiomyocytes required factors from human cardiomyocytes. How to coax these cells to form other clinically useful cells (eg, endothelial cells) and to fully functional cardiomyocytes remains a major challenge (4).

and pancreatic islet cells.<sup>10–13</sup> Unlike transcription factor-mediated reprogramming, the components of chemical cocktails often overlap substantially although the destination cell types differ greatly. Accordingly, the molecules used by Cao et al<sup>1</sup> have been used before. CHIR99021 is a GSK3 $\beta$  inhibitor that activates the canonical Wnt pathway and is present in most cell fate conversion cocktails leading to iPSCs, neurons, and now, cardiomyocytes.<sup>1,10–12</sup> A83-01 is an ALK4/5/7 inhibitor that blocks the Activin/Nodal branch of the transforming growth factor  $\beta$  pathway. Blocking A83-01 and other ALK inhibitors (RepSox, SB431542) have also been used in iPSC, neuron, and pancreatic islet cell reprogramming<sup>10,12,13</sup> and likely lead to chromatin remodeling conducive for the cardiac muscle program of gene expression.<sup>14</sup> Similarly, BIX01294 (a G9a histone methyltransferase inhibitor) and other epigenetic modulators are common components of reprogramming cocktails.<sup>8,10,12,13</sup> A direct hypothesis is that these common pathway/epigenetic modulators lead fibroblasts into a highly plastic state, which is poised to receive further instructions to activate the cardiomyogenic program. For practical applications, it is immensely important to define the conditions to exclude unwanted cell conversions, ie, teratomas, especially for therapeutic reprogramming in vivo where dose, timing, and stoichiometry will be hard to control.

### Lingering Challenge of Cardiomyocyte Maturation

After exposing human foreskin fibroblasts sequentially to 9C and cardiac induction medium, Cao et al<sup>1</sup> injected the cells into infarcted hearts of immunodeficient mice, where the cells expressed cardiomyocyte markers and showed organized sarcomeric structures after 2 weeks, demonstrating that 9C-treated cells are capable of forming cardiomyocytes in vivo. The chemically converted cells lacked an organized sarcoplasmic reticulum and transverse-tubule structures typical of adult cardiomyocytes, suggesting electric and mechanical

immaturity. Thus, the chemically converted cells seem to be similar to CMs derived from human PSCs, which resemble fetal far more than adult myocytes. In the absence of CASQ2, KCNAB1, CAV3, KCNJ2, KCNJ8, S100A1, KCND3, SLN, and CACNA1, the cells are likely to have poor calcium handling. Although physiological function was not fully characterized, their immature morphology suggests far less force generation than adult myocytes, poor calcium-induced calcium release, immature adrenergic responsiveness, automaticity, and proarrhythmic potential. Thus, we anticipate a shift of emphasis to establishing the chemical conditions for cardiomyocyte maturation in future endeavors, possibly by inducing or mimicking the function of cardiomyogenic transcription factors.

### Disclosures

None.

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