The All-Chemical Approach
A Solution for Converting Fibroblasts Into Myocytes
Yu Liu, Mark Mercola, Robert J. Schwartz

Conversion of Human Fibroblasts Into Functional Cardiomyocytes by Small Molecules
Cao et al

Converting cardiac fibroblasts to cardiomyocytes has been considered as a regenerative strategy for myocardial infarction and other disorders. Recently, Cao et al1 devised a 3-step protocol. Human foreskin fibroblasts were first treated with 9 compounds (9C: CHIR99021, A83-01, BIX01294, AS8351, SC1, Y27632, OAC2, SU16F, and JNJ10198409) 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. 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.

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, HP1, and 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. 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. 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,
and pancreatic islet cells. 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 have been used before. CHIR99021 is a GSK3β inhibitor that activates the canonical Wnt pathway and is present in most cell fate conversion cocktails leading to iPSCs, neurons, and now, cardiomyocytes. A83-01 is an ALK4/5/7 inhibitor that blocks the Activin/Nodal branch of the transforming growth factor β pathway. Blocking A83-01 and other ALK inhibitors (RepSox, SB431542) have also been used in iPSC, neuron, and pancreatic islet cell reprogramming and likely lead to chromatin remodeling conducive for the cardiac muscle program of gene expression. Similarly, BIX01294 (a G9a histone methyltransferase inhibitor) and other epigenetic modulators are common components of reprogramming cocktails. 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 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.

**References**


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