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 al.1 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 screenings 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 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.8 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 MESP1.9 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 of particular significance to cardiovascular medicine.

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 sarcomplasmic 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


The All-Chemical Approach: A Solution for Converting Fibroblasts Into Myocytes
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Circ Res. 2016;119:505-507
doi: 10.1161/CIRCRESAHA.116.309146
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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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