Reprogramming the Cardiac Field

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Pluripotent Stem Cells Induced From Mouse Somatic Cells by Small-Molecule Compounds
Hou et al

Reprogramming In Vivo Produces Teratomas and iPSC Cells With Totipotency Features
Abad et al

Two recent reports show that it is possible to reprogram somatic cells to a stem cell state using only a chemical cocktail or by inducing genes in tissues of engineered mice. These studies herald a time when cellular plasticity can be manipulated directly in the body to regenerate damaged tissues and organs. How do we go from these milestones to therapeutic heart regeneration?

Induced pluripotent stem cells (iPSCs) have revolutionized the stem cell field ever since their introduction by Takahashi and Yamanaka in 2006, as reviewed in Robinton and Daley. iPSCs can be generated from a variety of mouse and human somatic cells through lentiviral-mediated overexpression of pluripotency-related genes such as Oct4, Sox2, Klf4, and cMyc (OSKM). Although phenotypically comparable with embryonic stem cells in that they can give rise to most cell types in the body, the fact that iPSCs are generated from adult patient tissues means that their differentiated derivatives can be used to model congenital disorders or even as autologous cells for transplantation.

Reprogramming somatic cells to a pluripotent state has involved gene transfer to transiently express key pluripotency genes such as OSKM. The potential safety risks of genome modification have engendered concern and, consequently, have driven the development of nonintegrating methods for expressing OSKM and similar reprogramming cocktails.

More recently, several groups have succeeded in replacing ≥1 of the reprogramming factors with small molecules, but to date these approaches still required transfer of ≥1 gene or mRNA. In a recent article in Science, Hou et al described the screening of small molecules to replace OSKM and, in the end, attained full reprogramming of mouse somatic cells to pluripotency. Achieving a small molecule-only protocol marks an important milestone and, probably, also a peak in efforts to develop integration-free reprogramming methods because most have been easy to replicate and adequately serves the majority of applications. Nevertheless, a tremendous amount remains to be learned about the mechanisms whereby a somatic cell can be made pluripotent, reflected by the rather low incidence regardless of technique (0.001% to 1% of somatic cells).

The second recent article, by Abad et al, showed that it is possible to reprogram most organs and tissues to pluripotency in vivo through transgenic expression of OSKM in engineered mice. Two conclusions stand out. First, in vivo reprogramming drives formation of pluripotent stem cells in most organs and tissues in situ and, predictably, gives rise to teratomas. Second, and perhaps unexpectedly, the native milieu efficiently reprograms cells to a primitive stem cell state such that the iPSCs can form trophoderm. Normally, embryonic stem cells and in vitro-generated iPSCs do not efficiently yield extraembryonic lineages, indicating that they are not truly totipotent. Based on transcript profiles, embryonic stem cells and in vitro-generated iPSCs closely resemble pluripotent epiblast cells of the blastocyst; however as shown by Abad et al, in vivo iPSCs more closely resemble developmentally earlier morula-stage embryos. These results suggest that, after the initial reprogramming event, the cellular milieu might guide progression toward totipotency versus pluripotency.

Evidence is emerging that reprogramming occurs when OSKM initiates a stochastic phase followed by a meta-stable phase during which incorrectly or partially reprogrammed cells can arise. Finally, stable endogenous expression of Sox2 initiates a deterministic phase that culminates in the induction of Nanog and acquisition of pluripotency. Hou et al identified small molecules by systematic exclusion of individual reprogramming genes, screening for molecules that replaced the omitted gene. By meticulously doing this for each of the 4 OSKM reprogramming genes, they found a set of 7 small molecules that allowed full reprogramming, with an efficiency of ~0.2%, which is comparable with that with transgenic methods, reflecting an advance during previous attempts that always needed ≥1 factor. Although the chemically induced iPSCs overtly resemble transgene-induced iPSCs in terms of differentiation potential, there remains significant concern that the compounds might induce genes that adversely affect the physiological function of the differentiated derivatives of the iPSCs. On the contrary, a major advantage of the chemical biology approach is that it can be used to identify relevant targets of the small molecules, potentially revealing new insights into the reprogramming process such as commonalities and differences related to the somatic cell species and tissue of origin. This was not performed by Hou et al; however, 2 of the...
compounds, Valproic acid and DZNep, inhibit histone deacetylation and methylation, respectively, both of which might alter chromatin to make the transcriptional landscape conducive for pluripotency. Thus, probing the mechanisms for chromatin remodeling and identifying the underlying genes at the target loci might shed light on the network logic of reprogramming.

From a translational perspective, there does not seem to be a pressing basic scientific need to optimize iPSC generation further; commercial vendors already provide custom-made iPSCs and a limited range of differentiated derivatives. In addition, in situ iPSC generation is compromised by the predictable consequence of teratomas. Nevertheless, the Abad and Hou articles together herald a future for cardiology in which therapeutic reprogramming might be achieved directly in patient tissues, perhaps by bypassing pluripotency and proceeding directly to cardiogenic cell types.

Recent articles from Islas et al, Ieda et al, Qian et al, and Song et al have shown that it is feasible to generate cardiomyocytes and cardiomyogenic progenitors in vitro by directly reprogramming mouse and human fibroblasts with transcription factors such as Gata4, Tbx5, Mef2c, Mesp1, Myocardin, Ets2, and Hand2. Although these studies were inspired by Yamanaka’s discovery, direct reprogramming is a concept first introduced in the late 1980s when Tasscott and colleagues identified MyoD as a single gene that could convert fibroblasts into skeletal myoblasts. Introduction of ≥1 lineage-specific transcription factor into fibroblasts thus converts somatic cells from diverse lineages directly into the lineage of choice, which has now also been attained for several other cell types, including neurons and pancreatic β-cells. An exciting application of the direct reprogramming approach is its translation to the in vivo context. Although teratoma induction in the Abad article clearly illustrated that reprogramming in vivo is more efficient than in vitro, it also demonstrated remarkable plasticity of adult organs in response to reprogramming. Furthermore, initial reports indicate that it is possible to take the next step and directly reprogram cells in vivo to facilitate endogenous repair. In the pancreas, for instance, functional β-cells can be generated in vivo from exocrine cells by delivery of Ngn3, Pdx1, and Mafa, 3 β-cell–specific transcription factors. Similarly, a degree of myocardial restoration after infarction in the mouse was achieved by directly reprogramming resident fibroblasts into cardiomyocytes.

Clearly, direct reprogramming has exciting benefits and may surpass iPSC generation as a routine tool to study cardiac biology and disease in patient-specific cells, and may even be adapted for therapeutic regeneration. Cardiac direct reprogramming is, however, still in its infancy, and the underlying biology is far from understood. We think that following a chemical biology approach may expose how direct reprogramming works and may reveal why reprogramming differs in mice versus humans. For example, in human cells, cardiac genes can be induced, yet spontaneous action potentials are rare and no contraction is observed. Interestingly, initial findings in the mouse suggest that cardiomyocytes made by direct reprogramming can show considerably more mature electrophysiological properties than their iPSC-derived counterparts, as demonstrated by action potential measurements; however, it is not clear how or why such a mature state is attained nor why it is not achieved with human cells, at least in vitro. Nevertheless, it is at an early stage of development and we need a deeper theoretical understanding of the phenomenon, direct reprogramming to cardiomyocytes seems to have a promising future. Thus, iPSC technology continues to humanize cardiac disease research, not only by making it possible to recapitulate congenital disease in a cell culture dish but also by suggesting that therapeutic regeneration might be enhanced by direct conversion of nonmyocytes directly in the heart of the patient.

**Sources of Funding**

This work was supported by the National Institutes of Health grants (RO1HL11360) and RO1HL108176), the California Institute for Regenerative Medicine Training Grant (TG2-01162), and the Fondation Leducq.

**Disclosures**

None.

**References**


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Circ Res. 2014;114:409-411
doi: 10.1161/CIRCRESAHA.113.302946

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