Labor Pains of New Technology
Direct Cardiac Reprogramming
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Chronic heart failure primarily of ischemic origin still remains a major cause of morbidity and mortality. Because postnatal cardiomyocytes have little regenerative capacity, the current therapeutic approaches for heart failure are limited. Therefore, a new strategy to improve cardiac function must be established. The generation of cardiomyocytes from pluripotent stem cells is a promising source of cardiomyocytes for regeneration therapy because of the robust proliferation and capacity for differentiation of pluripotent stem cells. Pluripotent stem cells from the patients can be generated from their somatic cells by introducing reprogramming factors.1–4 This reprogramming technology simultaneously opened up a new avenue to generate not only pluripotent stem cells but also somatic cells. Thus far, successful direct reprogramming into neurons,5,6 pancreatic β-cells,7 hepatocyte-like cells,8 and cardiomyocytes9 has been reported.

The introduction of GMT converted >20% of fibroblasts into α-myosin heavy chain (MHC)-GFP-positive cells, of which 20% expressed cardiac Troponin T (cTnT). The induced cardiomyocytes exhibited spontaneous Ca2+ flux, electric activity, and beating. More recently, the same group showed that retroviral delivery of GMT failed to induce cardiomyocytes from mouse cardiac or dermal fibroblasts. The inclusion of GMT converted 35% of the fibroblasts demonstrated an approximately 250-fold increase in cTnT expression. Furthermore, no spontaneous action potential was observed in these cells, despite 22% of the cells showing voltage-dependent calcium currents. Thus, GMT appears to induce partial reprogramming into the cardiac lineage.

Because one of us (S.Y.) has laboratories at both the Gladstone Institute and Kyoto University, Japan, we are well aware of the reproducibility of GMT-mediated direct cardiac reprogramming in other Gladstone laboratories, as well as a laboratory in Japan that the first author (Ieda) started when he became an independent investigator. Why, then, did the technology work only partially in another laboratory? As Chen et al mentioned, potential differences in the genetic backgrounds of the mouse strains, the methods used for fibroblast isolation and cultivation, the cardiomyocyte-specific promoters used, and the method of virus production may account for these differences. These results indicate that the GMT-mediated cardiac reprogramming may still need further improvements to become a routine procedure in a wide range of laboratories.

In direct reprogramming, efficiency and robustness are crucial. During induced pluripotent stem (iPS) cell generation, the reprogrammed cells acquire a more proliferative character, which should accelerate epigenetic reprogramming.12 This acceleration leads the reprogramming cells to be in a more stable pluripotent stem cell state. On the other hand, during direct reprogramming into cardiomyocytes, the cell cycle rate of the reprogramming cells decreases. Therefore, to achieve robust and efficient reprogramming, powerful reprogramming factors might need to be introduced.

The selection of the transcription factors may be critical for the efficiency of reprogramming. In the case of iPS cell generation, the addition of Gli1 overexpression or knockdown of p53 led to the enhancement of reprogramming toward pluripotency.13–18 In cardiac direct reprogramming, a combination of GMT and other transcription factors may improve the reprogramming efficiency. In fact, Olson and his colleagues showed that GMT-mediated cardiac reprogramming is enhanced by another cardiac transcription factor, Hand2.19

Some embryonic stem cell–specific miRNAs can promote the reprogramming toward the pluripotency,20 and the combination of miRNAs can generate mouse and human iPS cells without overexpression of transcription factors.21,22 Recently, Jayawardena et al found that a combination of miRNAs 1, 133, 208, and 499 was able to induce direct cellular reprogramming of fibroblasts to cardiomyocyte-like cells in vitro and in vivo.23 Thus, recent studies have revealed that miRNAs can be powerful tools for reprogramming, without the need for transcription factors. It was noteworthy that transient transfection of synthetic mimics of mature RNAs was capable of reprogramming fibroblasts and that continuous upregulation of miRNAs was not necessary to switch the cell fate toward cardiomyocytes.

Small molecules such as histone deacetylase inhibitors, G9a histone methyltransferase inhibitors, or vitamin C and a hypoxic environment are effective to increase the reprogram-
Direct reprogramming holds great promise to provide an unprecedented treatment for cardiac diseases. The combination of GMT with other transcription factors, miRNAs, or small molecules, such as a JAK inhibitor, may increase the efficiency and robustness of reprogramming. The cardiac microenvironment may also improve the reprogramming efficiency and thus make the technology more applicable in situ. We expect to learn more about the molecular mechanisms underlying direct reprogramming and to make further technical advances in the coming years. Ultimately, we hope to see the technology help patients suffering from cardiac failure.

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**References**

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