Heart disease is the major cause of morbidity and mortality worldwide.1,3 The current therapeutic approaches for heart failure are limited because postnatal cardiomyocytes have little regenerative capacity. Therefore, a new strategy needs to be established to improve the cardiac function.

**Article, see p 1147**

Gene therapy is one of the most attractive new therapeutic strategies. Several kinds of cardiac gene therapy have so far been reported. Jeffrey M. Isner’s group reported that the administration of plasmid vectors encoding cDNA of the 165-amino acid isoform of vascular endothelial growth factor induced angiogenesis in patients with ischemic heart disease and with ischemic limbs.3,4 The effect of vascular endothelial growth factor gene therapy is mediated by the paracrine of cytokines (Figure, A).

Another type of gene therapy targets cardiomyocytes. The expression and activity of sarcoplasmic reticulum Ca\(^{2+}\) ATPase 2a (SERCA2a) have been observed to decrease in cardiomyocytes in a failing heart, and the overexpression of SERCA2a could restore the cardiac function in heart failure by improving calcium handling in the cardiomyocytes (Figure, B). Clinical trials for SERCA2a gene therapy were conducted for patients with heart failure, and positive results (Figure, B). Clinical trials for SERCA2a gene therapy were conducted for patients with heart failure, and positive results have been reported.5,6

A new strategy for gene therapy is to restore the number of target cells by direct conversion from other types of cells (Figure, C). Twenty-five years ago, Weintraub et al7 demonstrated that a single master transcription factor, MyoD, was capable of converting mouse fibroblasts into skeletal muscle cells. This first demonstration of cell fate conversion in mammalian cells was then applied in vivo.8,9 However, attempts to identify master factors for other cell lineages, including cardiac myocytes, were painfully unsuccessful.10

This situation suddenly changed after the demonstration of induced pluripotent stem cells; pluripotent state can be induced not by a single factor but by a combination of 4 transcription factors.11-14 It did not take long for other researchers to identify other combinations of transcription factors that can induce direct conversion to pancreatic β cells,15 neurons,16,17 hepatocyte-like cells,18,19 as well as cardiac myocytes.20-22

The first report of direct in vitro reprogramming into cardiomyocytes was published by Ieda et al.20 They demonstrated that the combination of GATA4, MEF2C, and TBX5 (GMT) was able to reprogram cardiac fibroblasts directly into cardiomyocytes in vitro. This cardiac direct reprogramming technology was reproduced with different factor combinations by other groups.21,22

Earlier this year, 2 groups reported in vivo conversion of fibroblasts into cardiomyocytes. Song et al22 reported that the injection of retroviruses encoding GMT and Hand2 converted β-galactosidase-expressing cardiac fibroblasts in Fsp1-Cre/Rosa26-LacZ mice into cardiomyocytes. Qian et al21 also reported that retroviral delivery of GMT in mice induced direct reprogramming of cardiac fibroblasts into cardiomyocytes. They used periostin-Cre/Rosa26-LacZ mice and Fsp1-Cre/Rosa26-LacZ mice, in which only descendants of the nonmyocyte population were β-galactosidase-positive and confirmed that the noncardiomyocytes were reprogrammed into cardiomyocytes. These reprogrammed cells revealed ventricular cardiomyocyte-like action potentials and a response to electrical stimulation, and electrical coupling with neighboring cells.

In this issue of *Circulation Research*, Inagawa et al24 reported another evidence of direct reprogramming into cardiomyocytes. In addition to the conventional retroviral vectors, they used a polycistronic retrovirus expressing GMT by a 2A system, which was previously reported to be useful for the efficient generation of induced pluripotent stem cells.25,26 Introduction of a polycistronic retrovirus to transduce 3 factors into fibroblasts generated more matured cardiomyocytes compared with introducing 3 separate vectors. The injection of polycistronic GMT (3F2A) converted around 1% of the infected cells. Although the conversion efficiency was almost the same as that of the conventional retroviruses, 30% of the converted cardiomyocytes showed cross striations in 3F2A-infected hearts, whereas 15% of the converted cells were striated in the conventional method. These findings suggested that efficient introduction of 3 genes by 3F2A resulted in generation of more matured cardiomyocytes.

The conversion efficiency from cardiac fibroblasts into cardiomyocytes was found to be 1%, and a large population of infected cells failed in the full conversion into cardiomyocytes and were thought to undergo only partial reprogramming. Do these partially reprogrammed cells finally revert to fibroblasts or remain in a partially reprogrammed cell state? Whether these partially reprogrammed cells play a role in the improvement or deterioration of the cardiac function is not known. A genetic tracing analysis of the reprogrammed fibroblasts might be informative to clarify the fate of reprogrammed cells. It is necessary to learn more about the molecular mechanisms and behavior of reprogrammed cells in cardiac direct reprogramming.

It is noteworthy that Inagawa et al24 observed no tumor formation in the treated mouse hearts. Inagawa et al24

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reported that most retrovirus-infected cells were removed by an immune response within 4 weeks in immunocompetent mice. In such cases, many supportive effects, including mechanical support by induced cardiomyocytes, will be lost after reprogrammed cells disappear. To obtain long-lasting effects, other types of gene delivery methods may be desirable for such clinical application.

Conversion efficiency is very important to achieve the clinical application of in vivo direct reprogramming technology. Another group reported that the in vitro conversion from adult fibroblasts into cardiomyocytes was very low. The combination of GMT with other transcription factors, microRNAs, small molecules, or other devices such as the 2A system, may increase the efficiency.

In this article, Inagawa et al reported that the polycistronic 2A system can be used for in vivo direct reprogramming and succeed in generating more matured cardiomyocytes in vivo. This polycistronic system might facilitate the application of other types of gene delivery methods which are less immunogenic or make no genomic integration. Finally, the advance of direct cardiac reprogramming technology could provide a new strategy for conducting gene therapy in patients with cardiac failure.

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