Heart disease is the major cause of morbidity and mortality worldwide. 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.

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. Clinical trials for SERCA2a gene therapy were conducted for patients with heart failure, and positive results have been reported by improving calcium handling in the cardiomyocytes. SERCA2a could restore the cardiac function in heart failure. Clinical trials for SERCA2A gene therapy were conducted for patients with heart failure, and positive results have been reported.

A new strategy for gene therapy is to restore the number of target cells by direct conversion from other types of cells. This first demonstration of cell fate conversion in mammalian cells was then applied in vivo. However, attempts to identify master factors for other cell lineages, including cardiac myocytes, were painfully unsuccessful.

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. It did not take long for other researchers to identify other combinations of transcription factors that can induce direct conversion to pancreatic \(\beta\) cells, neurons, hepatocyte-like cells, as well as cardiac myocytes.

The first report of direct in vitro reprogramming into cardiomyocytes was published by Ieda et al. 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.

Earlier this year, 2 groups reported in vivo conversion of fibroblasts into cardiomyocytes. Song et al reported that the injection of retroviruses encoding GMT and Hand2 converted \(\beta\)-galactosidase-expressing cardiac fibroblasts in Fsp1-Cre/Rosa26-LacZ mice into cardiomyocytes. Qian et al 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 \(\beta\)-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 al 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. 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 al observed no tumor formation in the treated mouse hearts. Inagawa et al...
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.27 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.

Sources of Funding
The authors were supported by the Lading Project of the Ministry of Education, Culture, Sports, Science and Technology, and the Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program) of the Japanese Society for the Promotion of Science.

Disclosures
S.Y. is a member without salary of the scientific advisory boards of iPierian, iPS Academia Japan, Megakaryon Corporation, and Retina Institute Japan. The other author has no conflicts to report.

References

Figure. Schematic presentation of 3 types of gene therapies.


Key Words: cardiomyocyte − direct reprogramming − transcription factor
An Emerging Strategy of Gene Therapy for Cardiac Disease
Yoshinori Yoshida and Shinya Yamanaka

Circ Res. 2012;111:1108-1110
doi: 10.1161/CIRCRESAHA.112.278820
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
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:
http://circres.ahajournals.org/content/111/9/1108

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/