Making Steady Progress on Direct Cardiac Reprogramming Toward Clinical Application

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Reprogramming of Human Fibroblasts Toward a Cardiac Fate
Nam et al
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A new report demonstrates direct cardiac reprogramming in human cells for the first time and points to the possibility of moving this technology toward clinical applications.

Direct reprogramming to somatic cells by forced expression of a combination of lineage-specific transcription factors or micro RNAs (miRNAs) has been demonstrated in a variety of rodent cell types, such as neurons,[1] neural stem cells,[2] hepatocyte-like cells,[3,4] β-cells,[5] and cardiomyocytes.[6–10] Three groups have reported the direct neuronal reprogramming of human fibroblast into neuronal cells.[11–13] Pang et al[11] demonstrated that the same 3 transcription factors used for mouse cells, Brn2, Ascl1, and Myt1l, are insufficient to induce functional neurons from human fetal fibroblasts, but that Brn2, Ascl1, and Myt1l plus the addition of NenroD1 can convert human fetal and postnatal fibroblasts into functional neurons. Yoo et al[13] also reported that a combination of neuronal transcription factors and 2 miRNAs, micro RNA (miR)-9/9* and miR-124, can efficiently convert human fibroblasts into functional neurons. These findings suggest that, compared with mouse cells, additional factors might be required to direct the reprogramming of human somatic cells into other lineage cells.

More recently, Nam et al[14] reported the direct reprogramming of human fibroblasts into cardiac-like cells (iCLMs). They first tested whether the same cardiac transcription factors, Gata4, Mef2c, and Tbx5 (GMT) or GMT+Hand2, which were previously reported to be useful for the direct reprogramming of mouse fibroblasts into cardiomyocytes,[6–8,10] could reprogram neonatal human foreskin fibroblasts into iCLMs. However, GMT and GMT+Hand2 both failed to induce cardiac marker expression in human foreskin fibroblasts. To determine the optimal combination of factors for the direct cardiac reprogramming of human fibroblasts, they examined the effects of additional factors, including other cardiac transcription factors and miRNAs, with GMT+Hand2. They found that a combination of Gata4, Hand2, Tbx5, Myocardin, miR-1, and miR-133 (6F) could convert human fibroblasts into iCLMs. The transduction of 6F induced ≈20% of human foreskin fibroblasts, ≈13% of adult human cardiac fibroblasts, and 9.5% of adult human dermal fibroblasts to express cardiac Troponin T 2 weeks later. Furthermore, spontaneous contractions were observed in a small subset of iCLMs derived from adult human cardiac fibroblasts 11 weeks later, but not from either human foreskin fibroblasts or adult human dermal fibroblasts.

In this study, they identified a combination of factors, 6F, that are capable of direct cardiac reprogramming from human fibroblasts. Interestingly, the transduction of Mef2c, which is one of the key factors used to reprogram mouse fibroblasts directly into cardiomyocytes,[6–8,10] with 6F significantly decreased the percentage of cardiac Troponin T$^+$ cells. They previously showed that miR-1-1/133a-2 and miR-1-2/133a-1 were downregulated in the hearts of mice lacking MEF2 expression and a MEF2-dependent upstream enhancer of the miR-1-1/133a-2 has been shown to regulate miR-1-1/133a-2 expression in cardiac and skeletal muscle in vivo.[16,17] Therefore, they mentioned that Mef2c regulates the expression of miR-1 and miR-133,[15] and that these miRNAs probably play an alternative role of Mef2c.

The precise molecular mechanism(s) underlying the direct cardiac reprogramming from fibroblasts is still unknown even in mice, and there is a possibility that there might be a mechanistic difference in the reprogramming process between mouse and human cells.

Regarding the direct reprogramming to somatic cells, the reprogramming efficiency and reproducibility are very important and often controversial subjects. A previous study by Chen et al[18] showed that forced expression of GMT in mouse tail tip fibroblasts and cardiac fibroblasts was insufficient to induce cardiac phenotypes. Nam et al[14] also showed that forced expression of a combination of miR-1, miR-133, miR-208 and miR-499, which were previously reported to be useful for the direct cardiac reprogramming of mouse fibroblasts,[9] failed to induce the expression of cardiac markers in mouse tail-tip fibroblasts. These findings suggest that an accumulation of slight differences among laboratories, such as the culture and isolation methods used for the fibroblasts, the fibroblast lines used or the method of virus production, might cause differences in the reprogramming efficiency and reproducibility. As indicated above, Nam et al[14] first reported the direct cardiac reprogramming of human fibroblasts. In their study, cardiac Troponin T$^+$ cells at 2 weeks and calcium transients at 4 weeks after the transduction of 6F were observed in ≈13% and ≈15% of adult human cardiac fibroblasts, respectively. However, spontaneous contractions were observed in only a few iCLMs derived from adult human cardiac fibroblasts. Many other researchers are

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also attempting to reprogram human somatic cells directly into cardiomyocytes, and thus, further improvements to achieve a high reprogramming efficiency are expected, and it is likely that the reproducibility of the method of Nam et al[14] will be demonstrated by another group in the near future.

The reprogrammed iCLMs from human fibroblasts were functionally immature. In the case of mouse cells, Inagawa et al[10] reported that the in vivo transduction of a polycistronic retrovirus vector expressing GMT separated by 2A peptides (3F2A) generated more mature cardiomyocytes compared with the in vivo transduction of GMT. In addition, some studies suggested that the in vivo environment, such as the presence of mechanical force and extracellular matrix and secreted proteins, might be more suitable for direct reprogramming with regard to the reprogramming efficiency, reprogrammed cell survival, and maturation.[5,7,8] The further development of gene delivery systems, such as polycistronic vectors, and the use of the in vivo environment might help to reprogram human fibroblasts into more mature cardiomyocytes.

In addition, the reprogrammed iCLMs from human fibroblasts were heterogeneous. In fact, the expression levels of cardiac and noncardiac genes in the iCLMs varied widely and only a small subset of iCLMs showed spontaneous contraction. It is supposed that partially reprogrammed cells exist in the population of reprogrammed cells, and such partially reprogrammed cells might cause arrhythmias in the heart. The authors mentioned that this heterogeneity of iCLMs is because of variations in the stoichiometry and levels of the expression of factors in individual cells and because of the heterogeneity of the original fibroblasts. In the future, improvements in the reprogramming efficiency and gene introduction methods may reduce the heterogeneity and thereby make the clinical application of this technology more feasible.

Attractive approaches, including direct cardiac reprogramming, have been developed in recent years with the goal of future cardiac regeneration therapy. Human pluripotent stem cells (PSCs), such as embryonic stem cells and induced PSCs, which can efficiently differentiate into cardiomyocytes after the addition of a combination of growth factors and are able to provide large amount of cardiomyocytes, are promising candidate cell sources. The transplantation of cardiomyocytes derived from human PSCs has already been reported to improve the cardiac function in rat and in swine infarction models.[19,20] In addition, Shiba et al[21] reported that human embryonic stem cell–derived cardiomyocytes can electrically couple and suppress arrhythmias in injured guinea pig hearts. However, many problems still remain to be resolved before the clinical application of human PSCs for cardiac disease. In particular, the elimination of undifferentiated PSCs to avoid tumor formation and the preparation of a large amount of cardiomyocytes derived from human PSCs are very important issues. The direct cardiac conversion from somatic cells is also a promising approach for cardiac regeneration therapy and may overcome the risk of tumor formation. Some groups have already reported the in vivo direct cardiac reprogramming of cardiac fibroblasts in the mouse heart.[7–10] In addition, Qian et al[7] and Song et al[8] revealed that the transduction of cardiac transcription factors in vivo improved the cardiac function and reduced fibrosis after myocardial infarction. Before the clinical application of this technology, the full effects of direct cardiac conversion and the safety of the transduction methods using defined factors must be demonstrated in large animal models. In addition, quantitative comparison of cardiomyocytes generated by direct cardiac conversion technology and PSC-derived cardiomyocytes will be required. Although direct cardiac conversion is still a developing technology, this new technology possesses great potential for future cardiac regeneration therapy.

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