The direct reprogramming of fibroblasts into functional cardiac myocytes is expected to bring new cell therapies for heart failure. Already, essential transcription factors for the reprogramming have been identified. However, several obstacles must be overcome before this technology benefits patients. In particular, there is a need to improve the efficiency of the reprogramming, especially with regards to acquiring mature cardiomyocytes. A recent article in Proceedings of the National Academy of Sciences suggests that the reprogramming can be enhanced with the addition of a kinase, Akt, to the induction protocol.

Many experimental cell therapies for heart failure aim to recover the number of functional cardiomyocytes lost. To achieve this purpose, direct conversion methods using transcription factors are being investigated. The first such method was reported by Weintraub et al, who converted fibroblasts into skeletal muscle cells using a single master transcriptional factor, MyoD. Almost 2 decades later, a combination of transcriptional factors was discovered to reprogram somatic cells into pluripotent stem cells in mice and humans. These discoveries demonstrated the high plasticity of cell fate and suggested that any given cell can be perturbed into another type given the right conditions. Accordingly, scientists have used different transcriptional factors to directly reprogram into many types of cells, including pancreatic beta cells, neurons, hematopoietic stem/progenitor cells, endothelial cells, and hepatocyte-like cell.

The first direct conversion to cardiomyocytes was reported by Ieda et al, who identified 3 transcriptional factors (Gata4, Mef2c, and Tbx5) as essential for the induction. The direct conversion of cardiac fibroblasts into cardiomyocytes in vivo was achieved by injecting viral vectors into hearts. An important advantage in the direct reprogramming of fibroblasts to cardiomyocytes over reprogramming that induces an intermediate stem cell state is the theoretically lower risk of tumorigenesis. Initially, the direct reprogramming efficiency was low (beating cells were only 0.01%–0.1%), but several methods, such as polycistronic vectors, other transcription factors, miRNAs, and small molecules, have since been devised and improved this number. Zhou et al has added to this work by considering a different strategy, which was to alter the intracellular signaling pathway with kinases. By screening a library of myristoylated kinases, they found that the addition of Akt1/protein kinase B to the above 3 cardiac transcription factors plus one more, Hand2, can significantly increase the conversion efficiency of mouse tail tip fibroblasts and cardiac fibroblasts into cardiomyocytes. Of further importance to cell therapies, Akt1 not only increased cardiac promoter-positive cells, but also increased cells that showed calcium influx and beating.

The authors examined several cellular characteristics, including morphology, metabolic profiles, expressions of sarcomeric genes, and mitochondrial membrane potentials, to conclude that their cardiac-like myocytes (iCMs) induced with the 4 transcription factors plus Akt were more mature than those induced with Gata4, Hand2, Mef2c, and Tbx5. Another factor regarding cardiomyocyte characteristics is multinucleation because cardiomyocytes derived from pluripotent stem cells tend to have significantly fewer multinucleated cells than those found in adult heart. Consistent with other maturity-related parameters, multinucleated iCMs induced with Gata4, Hand2, Mef2c, and Tbx5 plus Akt were more than those induced with Gata4, Hand2, Mef2c, and Tbx5.

In cardiomyocytes, Akt is known to affect a variety of biological activities, including cell survival, energy production, and response to mechanical stress. Transient activation of Akt results in physiological hypertrophy, whereas its prolonged activation induces pathological hypertrophy. In addition, Akt signaling is enhanced during in vitro cardiomyogenesis, and the inhibition of phosphoinositols 3-kinase/Akt signaling attenuates cardiomyocyte differentiation. The authors showed that the conversion efficiency into cardiomyocytes could be further improved by adding insulin-like growth factor 1, an extracellular activator of phosphoinositol 3-kinase/Akt signaling, to the protocol. In addition, downstream mediators of Akt, such as mTORC1 and Foxo3a, were shown to play a role. These 2 mediators have also been shown to regulate cardiomyocyte hypertrophy or energy metabolism, suggesting that they could play important roles in cardiomyocyte maturation.

Recently, Yamakawa et al reported that the addition of a combination of growth factors (fibroblast growth factor 2,
fibroblast growth factor 10, and vascular endothelial growth factor) to Gata4, Me2c, and Tbx5 enhanced reprogramming efficiency to iCMs through the phosphoinositide 3-kinase/Akt and the p38 mitogen-activated protein kinase pathways. These cytokines promoted the late stage of reprogramming, which facilitate the conversion of partially reprogrammed cells to fully reprogrammed functional iCMs with well-defined sarcomere structures. Given that enhancement in the late-stage reprogramming results in mature iCMs, both of these 2 papers highlight the role of intracellular signaling, including Akt, in the maturation of reprogrammed cardiomyocytes.

Continuous activation of the Akt signal is expected from a gene delivery strategy, which as explained above could cause pathological hypertrophy. Transient activation should be possible using the addition of cytokines, such as insulin-like growth factor 1 or fibroblast growth factor 2, fibroblast growth factor 10, and vascular endothelial growth factor promote cardiac reprogramming under defined conditions, downstream of AKT signaling.

Key to cardiac transdifferentiation by transcription factors is the complete rewriting of the original cellular identity to derive the new cell type. The results provided by Zhuo et al provide important insight on how the status of the intracellular signaling helps accomplish this conversion by revealing crosstalk between intranuclear and extranuclear factors. Optimizing the signaling pathways for efficient cardiac-direct conversion technology that are independent of genomic integrations should expedite the use of iCMs for clinical therapies.

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**References**


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