Repairing hearts with AKT

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Adult cardiomyocytes—the contractile units of the heart—have little regenerative capacity, and their loss, following ischemic injury and other insults, leads to cardiac dysfunction and heart failure, a degenerative condition with a poorer prognosis than many cancers. Despite intense efforts, there are presently no effective treatments for heart failure besides heart transplantation, itself limited by the number of organ donors and immunosuppressive therapies. Cell therapy has emerged as an attractive option for heart repair following myocardial infarction, which often progresses to heart failure. Over the past decade, many clinical trials have tested the ability of a variety of exogenous progenitor cells at repairing ischemic and failing hearts, but with little success; in most cases, cells transferred to the heart were unable to generate new cardiomyocytes (1). Alternative strategies, including in situ generation of cardiomyocytes at the infarcted area or in vitro reprogramming of cells into cardiomyocytes for organ grafting, are being explored.

Strategies to Generate New Cardiomyocytes

A promising avenue is the use of transcription factors to reprogram stem or somatic cells, such as fibroblasts, into beating cardiomyocytes. The discovery that transcription factor GATA4 cooperates with other cardiac transcriptional regulators to enhance cardiogenesis opened interesting avenues for heart repair (2–4). In the past few years, several studies showed that a combination of cardiac transcription factors, GATA4, MEF2C and TBX5 (GMT) or GHMT (GMT + HAND2) can be used to directly reprogram mouse fibroblasts into cardiomyocytes (5–8). Notwithstanding these impressive advances, several limitations still need to be overcome for this approach to realize its clinical potential. Chief among them is the modest efficiency of the reprogramming process and the need to achieve a more mature cardiac phenotype in these induced cardiomyocytes (iCMs). In PNAS, Zhou et al. show that addition of the protein AKT/protein kinase B to GHMT enhances the efficiency of reprogramming fibroblasts into iCMs, and significantly increases the number of cells displaying a mature cardiomyocyte phenotype (9). These findings represent important advances toward translating basic discoveries into regenerative therapies for heart repair.

Despite intense efforts in the last decade to improve stem cell-based therapies for myocardial repair, several issues remain to be resolved, such as the heterogeneity and immaturity of cardiomyocytes generated from stem cells, the risk of teratoma formation by contaminating induced pluripotent stem cells, and the poor retention of transplanted cells in the injured heart (10). The heart is a heterogeneous organ composed not solely of cardiomyocytes but also of many nonmuscle cell types, the majority of which are fibroblasts. During myocardial injury, resident fibroblasts migrate to the injured areas and form scar tissues to help maintain the heart's structural integrity, but they lack any contractile function. Cardiac fibroblasts are interesting candidates for direct reprogramming into cardiomyocytes because they are abundantly present at injured sites; they also derive from a common cardiogenic precursor pool as cardiomyocytes and express several cardiomyocyte genes. An alternative strategy for heart repair would therefore be to generate functional cardiomyocytes directly from fibroblasts without going through a stem cell stage.

The study by Zhou et al. opens important avenues to further elucidate the mechanisms and molecules involved in cardiomyocyte generation and maturation.

In the last 5 years, several approaches have been used to reprogram different types of fibroblasts such as mouse embryonic fibroblasts (MEFs), mouse tail tip fibroblasts (TTFs) and cardiac fibroblasts (CFs) into iCMs. For the past years, several groups have searched for optimal combinations of factors to improve the cardiac reprogramming efficiency using GMT with additional factors, including Mesp1, Hand1/2, Nkx2.5, Myocardin, Smarcd3, SRF, or microRNAs like miR-133 (reviewed in ref. 11). Although these studies suggested that direct reprogramming of fibroblasts to iCMs may hold great potential for cardiac repair, the functional properties, including calcium transients and spontaneous contractions, were observed in only a subset of iCMs, indicating that reprogramming into a functional cardiomyocyte was not yet achieved.

Zhou et al. (9) screened a kinase library for kinases that can enhance GHMT-dependent reprogramming. They found that adding AKT1 to GHMT (AGHMT) potentiates the reprogramming process of three different fibroblasts cell types (MEFs, CMs, and TTFs) into functional and mature cardiomyocytes. By isolating these three types of fibroblasts from αMHC-GFP transgenic mice, the authors found that AGHMT increased the number of αMHC-GFP$^+$ cells that express cardiac markers and have striated sarcomeres indicative of mature iCMs. To confirm that AGHMT-induced CMs have features of functional cardiomyocytes, the authors used a transgenic model expressing a conditionally active GFP calcium reporter, GCamp3, under the control of the αMHC promoter. AGHMT was able to convert fibroblasts into spontaneously beating cardiomyocytes with increased cellular calcium flux that were responsive to cardiac growth stimuli. This mature phenotype of iCMs was further validated with different biochemical and functional data, including increased ratio of Myh6/Myh7 expression, induced metabolic profile and mitochondrial membrane depolarization activity, as well as the presence of bi- or multinucleation without subsequent cytokinesis. Genome-wide analysis of gene expression using RNA-Seq showed that the gene expression pattern of AGHMT iCMs was very similar to that of adult mouse ventricular cardiomyocytes, albeit for the presence of additional fibroblasts markers. In an attempt to understand the mechanism of AKT1 action in this transdifferentiation process, Zhou et al. manipulated certain

Author contributions: H.K. and M.N. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 11864 in issue 38 of volume 112.

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downstream targets of AKT and assessed their effect on AGHMT's ability to induce CMs. Pharmacologic inhibition of mammalian target of rapamycin C1 or addition of Foxo3a attenuated AKT1 enhancement of the fibroblasts to iCMs conversion. However, AKT action seems to be independent of GSK3.

AKT Action in Cardiac Protection and Repair

Taken together, the data (9) strongly demonstrate that AGHMT drive fibroblasts to convert efficiently into fully reprogrammed iCMs. Although in vivo approaches in murine models of myocardial infarction will have to be carried out to confirm whether AGHMT leads to significant improvements to the ischemic heart, the study by Zhou et al. (9) opens important avenues to further elucidate the mechanisms and molecules involved in cardiomyocyte generation and maturation. The results presented are consistent with an earlier study that reported the beneficial effect of thymosin-β4, known to improve cardiac repair and activate AKT (12), in potentiating GMT-dependent cardiac reprogramming and improving heart function (13). The discovery that AKT signaling potentiates the cardiac reprogramming process is also consistent with the reported ability of AKT to promote the reprogramming of mouse primordial germ cells to an ESC-like state and, more recently, to generate induced pluripotent stem cells, at least in part, via the transcriptional activation of the pluripotent transcription factor Nanog (14, 15). The molecular mechanisms underlying the cardiac reprogramming activity of AKT1 in the context of GHMT remain to be elucidated. For example, is AKT acting through one or more of the GHMT factors or is it regulating a convergent synergistic pathway? Zhou et al. (9) verified the level of the GHMT transcripts and protein and found them unchanged. However, this does not exclude the possibility of AKT1-mediated post-translational modifications. AKT1 can phosphorylate HAND1 and alter its transcriptional and DNA binding activity in cardiomyocytes (16); whether AKT1 modulates the activity of any of the GHMT or their cofactors deserves to be analyzed.

Dissecting the action of AKT in cardiogenesis versus cardiomyocyte maturation will be especially interesting. Although AKT addition doubles the number of iCMs, it quadruples the number of mature myocytes. Zhou et al. (9) did not measure the effect of AKT on iCMs survival, a process that may participate in the observed AKT1 effects. AKT1 has been shown to increase cardiomyocyte survival following ischemia, to induce physiological hypertrophy, and to promote angiogenesis (17, 18). Whether these actions of AKT1 are mediated by components of the GHMT or by distinct pathways will be interesting to determine.

Notwithstanding the mechanistic uncertainties, the reported improvements in the number and mature phenotype of iCMs brought about by AKT1 constitute an important step forward for basic and translational science alike. The data reported by Zhou et al. (9) will no doubt stimulate further research into cardiogenesis and heart repair, and this will be beneficial to science and ultimately to patients suffering from ischemic heart disease.

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