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Direct Cardiac Reprogramming: From Developmental Biology to Cardiac Regeneration

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Direct Cardiac Reprogramming: From Developmental Biology to Cardiac Regeneration
Use of iPSCs to Generate Cardiac Myocytes and Repair Infarcted Myocardium
Genomic Stability Issues Associated With the Reprogramming Process
Differentiation of iPSCs Into Mature Myocytes

Shinya Yamanaka, Guest Editor

Direct Cardiac Reprogramming
From Developmental Biology to Cardiac Regeneration

Li Qian, Deepak Srivastava

Abstract: **Heart disease affects millions worldwide and is a progressive condition involving loss of cardiomyocytes. The human heart has limited endogenous regenerative capacity and is thus an important target for novel regenerative medicine approaches. Although cell-based regenerative therapies hold promise, cellular reprogramming of endogenous cardiac fibroblasts, which represent more than half of the cells in the mammalian heart, may be an attractive alternative strategy for regenerating cardiac muscle. Recent advances leveraging years of developmental biology point to the feasibility of generating de novo cardiomyocyte-like cells from terminally differentiated nonmyocytes in the heart in situ after ischemic damage. Here, we review the progress in cardiac reprogramming methods and consider the opportunities and challenges that lie ahead in refining this technology for regenerative medicine. (*Circ Res*. 2013;113:915-921.)**

Key Words: fibroblasts ■ heart diseases ■ myocytes, cardiac ■ reprogramming

Cardiomyocyte death is associated with diverse forms of heart disease, which is the leading cause of morbidity and mortality in industrialized countries. Myocardial infarctions are a major public health problem and affect >1 million Americans yearly. A tremendous loss of cardiomyocytes through apoptosis and necrosis underlies both acute and chronic myocardial infarction. To compensate for the death of cardiomyocytes in the injured area, scar tissue is formed by activated fibroblasts. In addition, heart diseases related to pressure overload, such as hypertension or valvular disease, involve the death of cardiac muscle cells, but the progression is slow and usually occurs during a long period of time. Human aging is associated with an irreversible loss of cardiomyocytes, which may account for the increased vulnerability of aged hearts to various risk factors.[1]

Unlike amphibian and fish hearts,[2] the human heart has limited regenerative potential. Although postnatal vertebrate cardiomyocytes undergo some degree of cell renewal, as suggested by transgenic mouse experiments[3–5] and a more recent human radiocarbon dating study,[6] the rate of cardiomyocyte renewal is low. A genetic fate map study involving mice indicated that the endogenous regenerative capacity of the adult heart, albeit limited, largely comes from the differentiation of cardiac progenitor cells rather than replacement by existing cardiomyocytes

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Nonstandard Abbreviations and Acronyms	
GMT	Gata4, Mef2c, and Tbx5
iCM	induced cardiomyocyte
iPSC	induced pluripotent stem cells
miRNA	microRNA

through cell division.[7,8] A major challenge moving forward is to identify such cardiac progenitor pools in the adult heart and promote their expansion and differentiation potential in vivo. In contrast, Porrello et al[9] reported the neonatal mouse heart has a remarkable regenerative capacity within the first 7 days of life, similar to that of the zebrafish heart. Genetic fate mapping suggests that myocyte proliferation is the main mechanism for this regeneration,[9] which is reminiscent of what has been found in the adult zebrafish heart.[10,11] Furthermore, in a recent report using stable isotope labeling with genetic fate mapping, in combination with multi-isotope imaging mass spectrometry, the authors concluded that pre-existing cardiomyocytes are the dominant source of myocyte renewal in the adult mouse heart with or without injury.[5] Cardiomyocyte proliferation has been reported to be enhanced by overexpressing cyclin D2[12,13] or administering factors, such as periostin,[14] fibroblast growth factor-1,[15] or neuregulin 1,[16] in mouse models of myocardial infarction. Most recently, addition of microRNA-199a and microRNA-590 also stimulated cardiomyocyte proliferation in vitro and in vivo.[17] Thus, there is increasing evidence that adult mammalian cardiomyocytes have the capacity to proliferate, but whether approaches to enhance this feature are sufficient to compensate for the functional loss of damaged myocardium awaits confirmation.

Another strategy to repair an injured heart is to supply new cardiomyocytes differentiated from multipotent cardiovascular progenitor cells or pluripotent stem cells, including embryonic stem cells and induced pluripotent stem cells (iPSC; reviewed extensively elsewhere).[18–21] Cardiomyocytes derived from pluripotent stem cells are a promising source of cells because of recent advances in improving the efficiency of human cardiomyocyte differentiation. However, poor survival, low maturation efficiency, and limited functional integration of engrafted cells are hurdles that must still be overcome. Introduction of adult progenitor cells in animal models has positive effects after myocardial infarction, but the cells do not seem to persist, pointing to a potential paracrine effect.[22,23] Clinical trials are underway to evaluate efficacy, with mixed results to date.[24]

Here, we review a newly emerging strategy for cardiac regeneration that involves reprogramming cells from the vast pool of nonmyocytes in the heart into new cardiomyocytes (Figure 1).[25] This approach builds on recent cellular reprogramming knowledge[26] and leverages >20 years of advances in cardiac developmental biology[20] to induce a cardiomyocyte-like fate in the adult heart. Cardiac reprogramming can be induced by a discrete set of factors but seems to have critical epigenetic blocks in vitro, which are significantly overcome on reprogramming of cells in their native environment in vivo.[25,27–30] We review how advances in cellular reprogramming and developmental biology led to our recent work in direct cardiac reprogramming and consider the future potential of this rapidly evolving approach to cardiac regeneration.

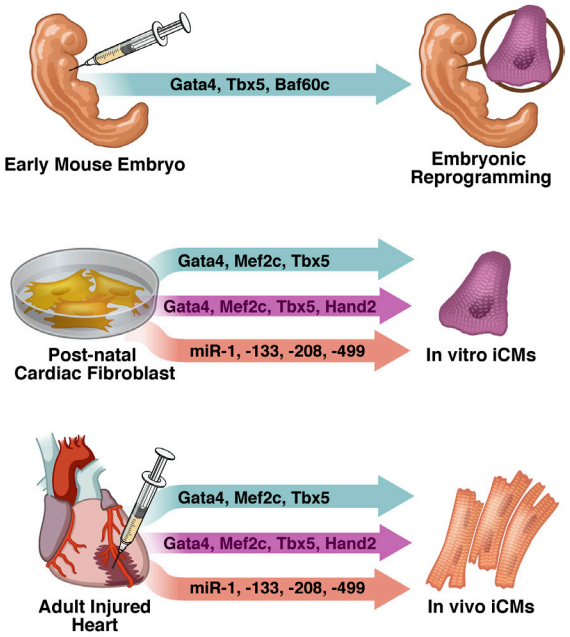

Figure 1. Approaches to direct cardiac reprogramming. Several methods for converting nonmyocytes to cardiomyocyte-like cells have been published and are summarized here. In the first example, injection of factors into the noncardiogenic mesoderm of embryonic day (E) 7.0 embryos resulted in ectopic beating cells (E8.5 embryos shown for simplicity). In vitro delivery of various cocktails resulted in primarily partially reprogrammed cells, whereas in vivo delivery yielded more fully reprogrammed cardiomyocyte-like cells. iCMs indicates induced cardiomyocytes.

Cellular Reprogramming

For decades, the concept in the field of developmental biology had been that cells, once terminally differentiated, were relatively fixed in their cell fate. This dogma was first challenged in the 1960s by the observation that a somatic cell can obtain totipotency through nuclear transfer into an enucleated frog egg.[31] Cloning of Dolly the sheep in 1997 by nuclear transfer revealed that the mammalian egg also had similar ability in reprogramming an adult nucleus to the embryonic state.[32] In the late 1980s and 1990s, further evidence suggested that cell fate conversion can take place by a more direct route. Blau et al[33] demonstrated that fusion of skeletal muscle cells with fibroblasts resulted in heterokaryons that induced conversion of fibroblasts into a skeletal muscle–like phenotype. Studies from the Weintraub laboratory subsequently demonstrated that fibroblasts could be

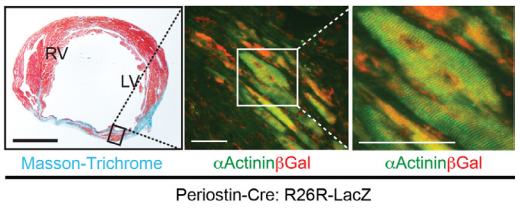

Figure 2. Direct conversion of cardiac fibroblasts into cardiomyocyte-like cells in vivo. Masson trichrome (**left**) and immunofluorescent staining for α-Actinin and β-Gal (**right**) in Gata4, Mef2c, and Tbx5-injected periostin-Cre:R26R-lacZ mouse heart 4 weeks after coronary ligation. Scale bars, 500 μm in the **left** panel, 50 μm in the **middle** and **right** panels. LV indicates left ventricle; and RV, right ventricle.

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converted into skeletal muscle cells in vitro with forced expression of the skeletal muscle master regulator gene *MyoD*, which encodes a basic helix-loop-helix (bHLH) domain-containing transcription factor.[34,35] Despite the race to identify individual transcription factors that could function to guide cell fate similar to MyoD for other lineages, including cardiomyocytes, the MyoD paradigm seemed to be an exception, rather than the rule. Meanwhile, studies in model organisms showed that the forced expression of individual master regulatory transcription factors containing homeobox domains could induce the formation of complex body structures, which is best exemplified by the induction of ectopic eye formation on the legs of *Drosophila* by overexpressing eyeless, the fly ortholog of Pax6.[36] The concept of cell fate conversion bypassing normal developmental lineage progression emerged but was still largely neglected with respect to the application of such concepts in regenerative medicine.

Takahashi and Yamanaka's[37] milestone publication in 2006 demonstrating the creation of iPSCs ushered in a new era of using cellular reprogramming in regenerative medicine.[38–40] Coexpression of 4 transcription factors (Oct4, Sox2, Klf4, c-Myc) was sufficient to reprogram fibroblasts to pluripotent stem cells that had the potential to develop into viable animals.[41,42] Abundant evidence has demonstrated that iPSCs can be differentiated efficiently into multiple cell types that someday could be used for regenerative therapies, disease modeling, and drug discovery. A key lesson from iPSC reprogramming was the recognition that a combinatorial code involving a discrete number of regulatory factors could be sufficient to induce cell fate change.[43,44]

Despite the difficulty in identifying MyoD-like factors for direct cellular reprogramming from one terminally differentiated adult somatic cell directly into another without taking a detour back to pluripotency, the iPSC experience raised the possibility of a combination of regulators that could together induce cell fate change or transdifferentiation. This reprogramming strategy was first demonstrated by Zhou et al,[45] who directly converted exocrine pancreatic cells into insulin-producing endocrine cells in the mouse pancreas with the transcription factors Ngn3, Pdx, and Mafa. Similarly, we showed that cardiac and dermal fibroblasts could be reprogrammed into cardiomyocyte-like cells by a combination of 3 transcription factors, Gata4, Mef2c, and Tbx5.[25,27] The direct reprogramming of adult fibroblasts to neuronal-like cells was also achieved in vitro with forced expression of a combination of transcription factors[46,47] or microRNAs (miRNAs),[48] and similar observations were made for conversion to the hepatocyte lineage.[49,50] Unlike direct reprogramming with multiple factors, Szabo et al[51] showed that combining the expression of only Oct4 with administration of select cytokines can reprogram human fibroblasts into hematopoietic progenitors, whereas transient expression of iPSC reprogramming factors, followed by Jak/Stat inhibition, resulted in the emergence of cardiomyocytes.[52] The advances in cardiac reprogramming are considered in more detail.

Discovery of Transcription Factor–Based Direct Cardiac Reprogramming

During the past 20 years, developmental biology studies have revealed complex and intertwined networks of signaling pathways, transcription factors, and miRNAs that regulate the formation and function of the heart.[20,53,54] The networks are self-reinforcing, with layers of positive and negative feedback loops. Transcription factors often function in common complexes, and human mutations that disrupt their interaction can lead to similar forms of heart malformations as seen with mutations in GATA4 and TBX5.[55] In 2009, Takeuchi and Bruneau[56] demonstrated that overexpression of Gata4, Tbx5, and the interacting chromatin remodeling protein, Baf60c, converts noncardiogenic mesoderm into beating cardiomyocytes in the embryo by a mechanism involving the induction of Nkx2-5 by Gata4 and Baf60c.

The adult heart has many cell types within the organ that are normally noncardiogenic. The majority of these are cardiac fibroblasts, which comprise >50% of cells in the heart and are derived from an extracardiac structure known as the proepicardial organ.[57] Fibroblasts play an important structural and paracrine role supporting the neighboring myocytes.[58] Upon injury, cardiac fibroblasts are activated and migrate to the site of injury to create scar tissue that replaces dead myocardium. As a result of the abundance of resident cardiac fibroblasts, the ability to reprogram such cells in situ would represent a powerful approach for regenerating myocardium.

We leveraged the abundant knowledge of cardiac developmental biology to attempt reprogramming of adult somatic cells into cardiomyocyte-like cells.[25] Despite the desire to ultimately reprogram in vivo, we established an in vitro assay to discover the minimal cocktail of factors that could convert noncardiomyocytes in the heart into a more cardiomyocyte-like phenotype. We generated transgenic mice containing the EGFP reporter downstream of the cardiomyocyte-specific αMHC (Myh6) promoter[59] and isolated the EGFP-negative nonmyocyte population, which consisted largely of fibroblasts. On retroviral introduction of 14 transcription factors and a pool of miRNAs, neonatal αMHC-EGFP$^-$ cells activated the expression of αMHC (Myh6) and the αMHC-EGFP reporter, which allowed quantitative analysis by fluorescence-activated cell sorting. The miRNAs in this setting were dispensable. Through serial deletion of one transcription factor at a time, we narrowed the required reprogramming factors to the following 3: Gata4, Mef2c, and Tbx5 (GMT). The GMT cocktail was sufficient to induce green fluorescent protein (GFP) expression in ≈15% to 20% of the cells, which we termed induced cardiomyocytes (iCMs). However, the majority of cells were partially reprogrammed, with 5% of the total infected cell population expressing additional cardiac markers, such as cardiac Troponin T (cTnT), and assembling sarcomeric structures; furthermore, only ≈0.5% of the αMHC-EGFP$^+$/cTnT$^+$ cells were capable of beating.

Despite the low percentage of fully reprogrammed cardiomyocytes, genome-wide transcriptome studies of the αMHC-EGFP$^+$ cells (≈15% of infected cells) showed that the partially reprogrammed population induced a broad cardiac transcriptional program involving hundreds of genes and also silenced the fibroblast transcriptome. Furthermore, the epigenetic status of iCMs was similar to neonatal endogenous cardiomyocytes at loci examined, and the reprogramming event was stable, and did not require ongoing expression of GMT. Notably, the GMT cocktail also reprogrammed tail-tip dermal fibroblasts, albeit with lower efficiency, suggesting that the presence of a cardiac progenitor pool was not necessary for the presence of iCMs.

Interestingly, the more fully reprogrammed iCMs had action potentials that were most similar to adult ventricular myocytes. This observation was in contrast to the relatively immature

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electric activity noted in embryonic stem cell–derived or induced pluripotent stem cell–derived cardiomyocytes. Using a Cre-based strategy, we found that iCMs rarely expressed Mesp1 or Isl1, markers of early cardiac progenitors, during the process of cardiac reprogramming.[25] This suggested that the reprogramming event represented a direct conversion from one adult cell type to another rather than traversing through a progenitor stage. The rapidity of initial conversion and the more mature electrophysiology observed in iCMs are consistent with this interpretation.

In Vivo Direct Cardiac Reprogramming

The relatively poor quality and low efficiency of in vitro cardiac reprogramming might be because of the lack of a natural environment for cardiomyocytes on plastic dishes. Because the objective of this strategy was to harness the endogenous cardiac fibroblasts within the organ for regeneration without requiring the use of cell-based therapy, we attempted to deliver GMT in vivo after ischemic injury and to convert nonmyocytes to cardiomyocyte-like cells. Classic genetic lineage -tracing studies with periostin-Cre, Fsp1-Cre, and αMHC-MerCreMer were performed to demonstrate that dividing nonmyocytes infected by retroviruses could be converted into iCMs[27] during a period of 4 weeks. Careful studies demonstrated that the newly born myocytes arose from in vivo conversion rather than leaky Cre expression or cell–cell fusion. Importantly, intermediate stages of reprogramming to iCMs were identified and characterized at varying time points after retroviral infection, further supporting the idea of a progressive reprogramming process.

In vivo–derived iCMs developed many characteristics of endogenous cardiomyocytes. They were binucleate, assembled sarcomeres and had cardiomyocyte-like gene expression by 4 weeks after infection with GMT. Furthermore, single-cell analyses by patch clamp technology revealed that 50% of in vivo–derived iCMs closely resembled endogenous cardiomyocytes with beating on electric stimulation and ventricular cardiomyocyte-like action potentials. The markedly improved quality of reprogramming in vivo compared with in vitro might have been because of signals from the microenvironment, exposure to the extracellular matrix, or influence of mechanical forces while reprogramming. Importantly, we found evidence for electric coupling of the in vivo reprogrammed iCMs with endogenous cardiomyocytes and other iCMs. In vivo delivery of GMT intramyocardially decreased scar size and attenuated cardiac dysfunction after coronary ligation, as assessed by MRI and echocardiography. As expected, the cardiomyocytes within the scar area of GMT-treated mice represented newly born iCMs as determined by lineage tracing experiments (Figure 2). The beneficial effects of GMT were enhanced with the addition of the proangiogenic and fibroblast-activating factor, thymosin β4, which independently promotes cardiac repair.[60,61]

In a similar study, Song et al[28] replicated the findings of Ieda et al[25] with GMT and demonstrated that the addition of a bHLH domain–containing transcription factor, Hand2, could increase the efficiency of cardiac reprogramming in vitro. Hand2 was initially discovered in a search for a cardiac MyoD-like bHLH protein[62] and is essential for a subset of cardiac progenitors,[63–65] but is not sufficient to induce the cardiac phenotype. Importantly, Song et al[28] also showed direct conversion from nonmyocyte to myocytes in vivo by retrovirally transducing fibroblasts with GMT plus Hand2 into hearts after coronary ligation. These in vivo–induced cardiac-like myocytes were similar to the endogenous cardiomyocytes based on their gene expression, sarcomere structure, and electrophysiological features. Lineage tracing with Fsp1-Cre, an inducible Tcf21-iCre, and αMHC-MerCreMer demonstrated that the origin of the iCM-like cells was likely cardiac fibroblasts. This observation was further supported by the evidence that the genetically prelabeled old myocyte pool was diluted by these newly born myocyte-like cells. Along with the emergence of new cardiomyocyte-like cells, improved heart function and reduced scar size were observed.

A third successful example of in vivo cardiac reprogramming was reported by Inagawa et al.[30] The authors used the GMT cocktail and retroviral delivery method. Different from Qian et al[27] and Song et al,[28] immunosuppressed mice were used in an attempt to promote the survival of viral-transduced cells. Reprogramming with these nude mice resulted in more iCMs with well-defined sarcomere structure. Furthermore, to improve the transduction of GMT in vivo, Inagawa et al[30] generated a polycistronic retrovirus expressing GMT at near-equimolar levels from the same promoter using self-cleaving 2A peptides that resulted in a better reprogramming efficiency. Although the global outcome of GMT introduction in this system was not clear, use of a 2A polycistronic vector and immunosuppressed mice further refined the in vivo reprogramming technology.

These 3 studies provide compelling evidence that the abundant nonmyocyte pool in the heart, largely composed of fibroblasts, can be transdifferentiated into new cardiomyocyte-like cells in vivo after injury, resulting in regeneration of myocardium and improved cardiac function. The functional improvement can be partially explained by new myocytes that increase force generation, but a fundamental alteration in the nature of scar-producing fibroblasts could also account for part of the reduction in scar size and improvement in cardiac output.

Alternative Strategies for Cardiac Reprogramming

The strategy involving serial deletion of 1 factor at a time can yield potential combinations of factors for reprogramming but, because of the complex interactions between networks, may not necessarily yield the optimal combination. To address this, Protze et al[66] screened 120 triplet combinations of 10 important developmental cardiac transcription factors expressed by lentiviruses in mouse embryonic fibroblasts for their ability to induce a myocyte-like phenotype. Rather than measuring the activation of a single reporter, they assayed expression of a panel of cardiac genes by quantitative polymerase chain reaction. In this screen, myocardin, Mef2c, and Tbx5 comprised the optimal combination to convert fibroblasts into myocyte-like cells, determined by gene expression, sarcomere formation, and ion channel activity, suggesting multiple combinations may exist for efficient cardiac reprogramming. Protze et al[66] also performed a time-course experiment and showed a progressive process involved in direct cardiac reprogramming in which a more complete cardiac phenotype arose over time.

miRNAs have important roles in cardiomyocyte decisions and are often regulated by the major cardiac transcription factors and, in turn, titrate the dosage of the key transcriptional networks.[54,67] In particular, miR-1, the most abundant cardiac miRNA, promotes

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muscle gene expression and regulates many aspects of cardiac biology.[68–71] Recently, Jayawardena et al[29] showed that a combination of 4 muscle-specific miRNAs (miR-1, miR-133, miR-208, miR-499) was sufficient to transdifferentiate mouse fibroblasts into cardiomyocyte-like cells in vitro and in vivo. Remarkably, in the presence of a Janus kinase (JAK)-1 inhibitor, miR-1 alone was sufficient to reprogram the cardiac fibroblasts.[29]

Although many groups have now successfully reprogrammed fibroblasts to cardiomyocyte-like cells in vitro, others have struggled,[72] highlighting the challenges to easily mastering this technique. The use of fresh, nonsenescing fibroblasts, high titers of viruses expressing the reprogramming factors, and careful culture conditions are among the variables involved in achieving successful reprogramming.[73,74] Future work on standardizing the conditions and improving the conversion efficiency in vitro will be necessary to make this a routine procedure in many other laboratories.

Reprogramming Human Fibroblasts Toward the Cardiomyocyte Fate

In order to advance cardiac reprogramming technology, it will be important to translate the knowledge gained from studies using the mouse system into human cells. Several recent studies reported the failure of GMT or GMHT to convert fibroblasts to cardiomyocyte-like cells, but each described overlapping but distinct combinations of factors that could push human fibroblasts into a more cardiomyocyte-like state.[75–77] Nam et al[75] reported that a combination of 4 transcription factors (Gata-4, Hand2, Tbx-5, and Myocd) and 2 miRNAs (miR-1 and miR-133) could reprogram up to 20% of human fibroblasts into cTnT-expressing cells. Upregulation of cardiac genes and downregulation of fibroblast genes was observed, and some cells developed calcium transients with observation of rare beating cells. However, many cardiac genes failed to be upregulated, suggesting the switch from a fibroblast-like phenotype to cardiomyocyte-like phenotype is partial. Similarly, Fu et al[76] found that addition of MESP1 and myocardin to GMT, along with a nuclear hormone receptor, ESRRG, could induce gene expression shifts in human fibroblasts to a similar extent as GMT in mouse cells in vitro. Approximately 20% of these cells developed calcium transients, and some had action potentials similar to human pluripotent stem cell-derived cardiomyocytes. Wada et al[77] also found that addition of MESP1 and Myocardin to GMT could reprogram human fibroblasts into cells that had many properties of human cardiomyocytes. Thus, while human cells appear more resistant to conversion, various combinations of the core transcriptional machinery are able to shift cells considerably toward the cardiomyocyte state, although further improvements may be necessary. Whether the current combinations of reprogramming factors result in more complete reprogramming in vivo, similar to the experience in mouse cells, await studies in large animals, including non-human primates.

Challenges and Future Directions

Despite the excitement and potential of direct cardiac reprogramming technology, several challenges remain, as would be expected for any new technology. These challenges include developing a mechanistic understanding of the reprogramming process, improving efficiency in mouse and human cells, enhancement or replacement of genes with drug-like molecules, and performing in vivo efficacy and safety in large animals. Such efforts are underway in many labs, and the coming years should rapidly yield advancements in this nascent technology for regenerative medicine.

The low conversion rate of fibroblasts to fully reprogrammed iCMs in vitro is a major challenge for deciphering the mechanism of reprogramming. The relative inefficiency in vitro is not surprising, considering the iPSC reprogramming efficiency rate (0.01%–0.1%), which is likely because of major epigenetic barriers that cells cannot easily overcome.[78] Unlike iPSCs, reprogrammed cardiomyocytes rapidly exit the cell cycle, making efficiency a much bigger concern. Understanding how progressive epigenetic and transcriptional changes occur temporally during direct cardiac reprogramming is an important first step toward overcoming these hurdles. As highlighted from 2 recent genome-wide epigenetic studies of embryonic stem cell (ESC)-derived cardiomyocytes,[79,80] temporal alterations in chromatin structure patterns lead to activation of key genes associated with heart development and function at distinctive differentiation stages. Such mechanisms might be informative for cardiac reprogramming. Second, methods aiming to improve delivery efficiency, such as the use of a polycistronic vector[30] to control for homogenous reprogramming gene expression, nanoparticle techniques for specific and efficient targeting, immunosuppression to promote survival of transduced cells,[30] the administration of thymosin β4 to mobilize and activate fibroblasts,[60] and use of VEGF to promote angiogenesis,[81] may increase the final number of reprogrammed iCMs. Third, techniques that enhance the maturation of cardiomyocytes should be used to promote the progression of iCMs from immature to mature stages. These include supplying the cells with cytokines at defined stages, altering the expression pattern of certain ion channel regulators, exposing cells to mechanical forces, and providing extracellular matrix and endothelial cells. Finally, small-molecule and secreted factor screens geared toward increasing iCM numbers and quality should be performed to identify externally administered factors that promote the reprogramming process.

Approaches to improve efficiency in vitro will aid in mechanistic understanding of the reprogramming process and may ultimately allow use of direct reprogramming to model human disease. Although current technology does not generate sufficient numbers of fully reprogrammed iCMs for disease modeling studies, improvements in efficiency will be valuable because iCMs seem to achieve electric maturation that is more similar to ventricular myocytes, particularly when reprogrammed in vivo, and this has been difficult to achieve with ESC-derived or iPSC-derived cardiomyocytes. Generation of sufficient numbers of fully reprogrammed cells in vitro would also be valuable for drug toxicity studies and drug screening.

In contrast, current iCM technology is quite efficient for in vivo reprogramming. Thus, harnessing the vast endogenous pool of noncardiomyocytes seems like a viable approach to regenerate heart muscle without cell-based therapy. Although this would require a gene therapy approach ideally using nonintegration vectors such as adeno-associated viral vectors, a reasonable regulatory path exists for virally mediated gene delivery, with scores of Food and Drug Administration–approved trials underway and the recent approval of a gene therapy drug

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for lipoprotein lipase deficiency in Europe. Future identification of small molecules or secreted proteins that could replace each transcription factor, as has been performed for iPSC reprogramming,[82,83] may allow an alternative to gene therapy. It is possible that in vivo reprogramming of cells to regenerate damaged tissue will serve as a new paradigm for many human diseases, and the lessons learned in the cardiac area will be applicable to strategies to realize this dream in other tissues. Although many challenges lie ahead in advancing this nascent technology, the opportunities and the potential benefits are significant, and we are confident that the field will continue to push this technology further in the years ahead.

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