Cellular Reprogramming

A New Avenue to Cardiac Regeneration?

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The hearts of lower vertebrates have remarkable regenerative capacity.[1] Neonatal mouse hearts are also capable of repairing damaged myocardium; however, this capacity is lost barely 2 weeks after birth.[2] The human adult myocardium is replenished throughout life,[3] and studies suggest that this occurs as a result of division of existing cardiomyocytes,[4] with a possible contribution from resident adult cardiac stem cells.[5,6] Combined, these findings raise the prospect that it may be possible to activate the endogenous regenerative reserve of the heart to restore function in failing hearts.

Complementing this approach, new genetic reprogramming technologies, based on the pioneering work by the 2012 Physiology and Medicine Nobel laureates Gurdon[7] and Yamanaka,[8] have shown promise for the repair of myocardial damage. In this context, 4 recent articles[9–12] demonstrated that differentiated adult fibroblasts can be genetically reprogrammed into cardiomyocyte-like cells, termed induced cardiomyocytes (iCMs), raising the possibility that direct genetic reprogramming may have therapeutic applications. Given that fibroblasts constitute ≈40% of cells within the adult heart, if efficient conversion rates could be achieved, this may yield the billions of cardiomyocytes required to replenish the heart after an ischemic event. Crucially, the reprogramming process occurs in postmyocardial infarct heart,[10,11] as well as the laboratory dish. This finding raises the exciting possibility that human cardiac fibroblasts may be amenable to treatments designed to alter cellular identity in vivo. In this commentary, we discuss the reprogramming strategy as an alternative to current cellular-based approaches to myocardial regeneration (Figure).

Clinical Demand Is Driving Attempts at Cardiac Regeneration

As a consequence of progressive improvements in survival from acute myocardial infarction,[13] cardiac failure of ischemic origin is rising in prevalence. The primary focus of policy makers has, appropriately, been on primary prevention of atherosclerosis and on prompt and suitable treatment of acute coronary syndromes.[14] However, there remains an unmet clinical need for a large number of individuals with poor left ventricular function after myocardial infarction.[15] Despite availability of effective pharmacologic[16–18] and device[19] therapies for ischemic left ventricular dysfunction, the fact remains that left ventricular necrosis heals by scar tissue rather than replacement by new contractile myocardium. This is driving intense research activity aimed at augmentation of myocardial regeneration.

Trouble With Cellular Therapy

To date, clinical trials of potential regenerative therapy have focused on administration of various cell preparations (Table 1). These have included administration of bone marrow–derived cells,[20–25,27,28,32–34] skeletal muscle myoblasts,[29,35–37] mesenchymal stem cells,[38] cardiosphere-derived cells,[30] and c-kit–positive resident myocardial cells.[31] With the exception of skeletal muscle myoblasts, administration of cells has been safe, but efficacy has been modest at best. Despite the rapid progress to clinical trials, several key barriers remain, which may restrict the clinical use of these injected cell types as a potential regenerative therapy. These barriers can be divided into 2 major classes: (1) cardiac-specific problems and (2) generic difficulties common to all cellular therapies (eg, immunologic compatibility, delivery method). These are briefly outlined below and represented in Figure (A).

Cardiac-Specific Problems

A frequent observation is that myocardially injected cells display both poor viability and engraftment. The majority of injected cells are dead within 24 hours[39]; various studies suggest that somewhere in the order of 6% to 8% of injected cells are viable after 24 hours.[40,41] Presumably this relates to injection of cells into a hostile ischemic environment and loss of cell–cell interactions during suspension of cells for injection. The number of viable cells seems to decline rapidly and progressively within 24 hours too. In fact, after intramyocardial injection of molecularly labeled cells, there was no evidence of any viable transplanted cells remaining after 8 weeks, as assessed by bioluminescence imaging.[41]

A second potential complication of intramyocardial cell administration is the possibility of poor electric integration with host myocardium. Even cells that do survive and engraft may form islands of electrically isolated cells, with the potential to form the anatomic substrate for re-entry circuits with

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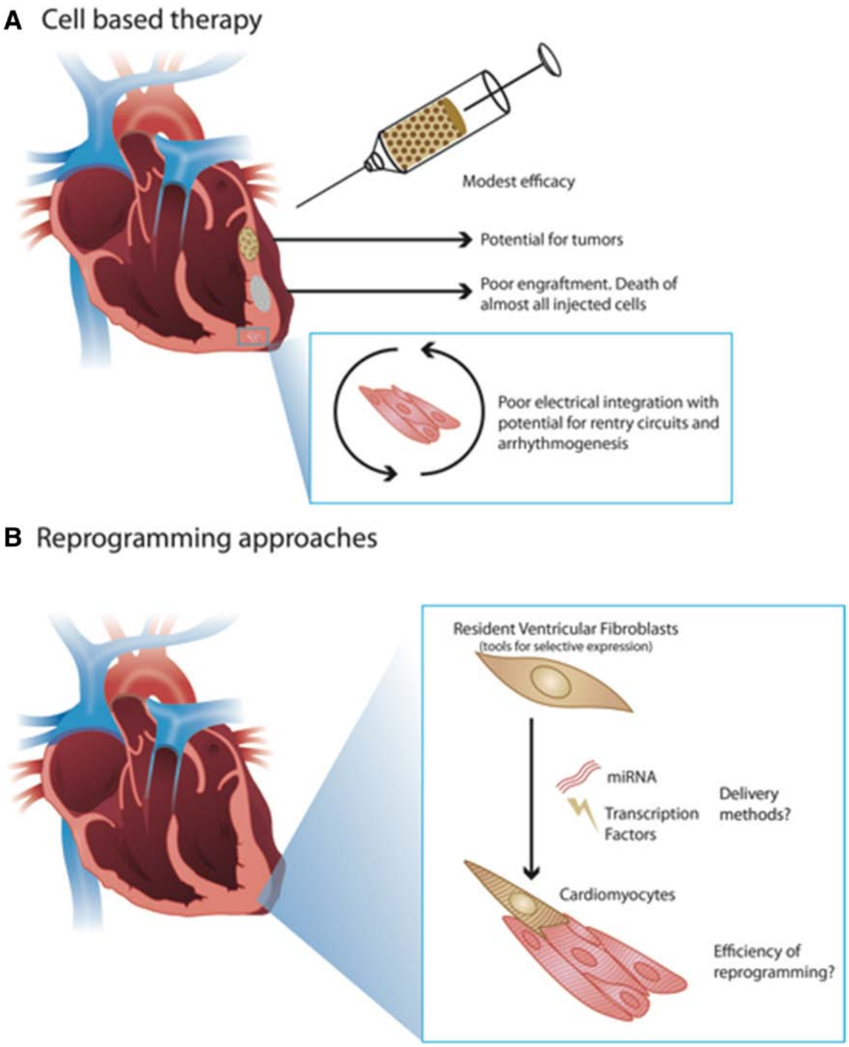

Figure. A genetic reprogramming strategy as a potential alternative to cell-based attempts at myocardial regeneration. **A**, Potential issues with cell administration to regenerate cardiac function include modest efficacy, poor engraftment, potential for tumor formation, and poor electric integration of injected cells. **B**, Recent demonstration that fibroblasts can be genetically reprogrammed to cardiomyocytes using defined transcription factors or microRNAs (miRNA) may represent an alternative approach to inducing meaningful myocardial regeneration.

attendant risk of ventricular arrhythmia. This danger signal was, in fact, observed during human trials of skeletal muscle myoblasts,[35] in which the treatment group experienced higher rates of ventricular tachycardia than the placebo-treated group. Reassuringly, however, recent xenografts of human embryonic stem–derived cardiomyocytes into guinea-pig hearts did seem to be electrically integrated.[42]

Finally, most injected cell types do not actually adopt a cardiomyocyte phenotype. Despite initial reports that bone marrow–derived cells could transdifferentiate into cardiomyocytes,[43] it seems that this is unlikely to be the case to any appreciable extent.[44,45] Thus, regeneration of the damaged myocardium is unlikely to be because of the efficient and robust differentiation of the cell types to date used for therapy. Indeed, the evidence suggests that for bone marrow mesenchymal stem cells[46] and cardiosphere-derived cells,[47] the dominant mode of action of injected cells is paracrine in nature.

Other Impediments to Cellular Therapy

The capacity to generate meaningful cardiomyocyte cell numbers for myocardial regeneration may be restricted to a few cell types. Attention has naturally turned to truly pluripotent cells—embryonic stem cells[48] or induced pluripotent stem cells[8]—for use as agents of myocardial regeneration because by definition such cells have the potential to differentiate into cardiomyocytes. In addition, induced pluripotent stem cells would circumvent the multiple ethical, legal, and other regulatory hurdles to the use of embryonic stem cells. If used in an autologous fashion, induced pluripotent stem cells could also sidestep the issue of immune rejection. However, intracardiac injection of induced pluripotent stem or embryonic stem cells into immunodeficient hosts results in teratoma formation.[49] Indeed, intrinsic to such cells is the danger of tumor formation if the cell preparations derived from them are injected without sufficient purification to remove every last undifferentiated cell.

Another potential issue is that ex vivo expansion of cells carries potential risks. Extended periods in cell culture are required for several cell preparations under evaluation such as cardiosphere-derived cells and c-kit resident cardiac cells. This introduces the potential for epigenetic changes, chromosomal abnormalities, and bacterial or viral contamination. The laboratories that spearhead clinical development of such cell preparations are cognisant of these potential risks and are at pains to establish and comply with exacting conditions for cell preparation using Good Manufacturing Practice. Nevertheless, epigenetic changes and minor genetic alterations are difficult to efficiently monitor using current technologies.

Table 1. Clinical Trials of Various Cell Types and Outcomes

Cell Type	Trials	Results of Trial, Benefits, and Limitations
Mononuclear cells (circulating or bone marrow derived)	BOOST[20,21]	Improved global LVEF at 6 mo Benefit no longer present at 18 mo
	ASTAMI[22]	Reduction in infarct volume No improvement in LVEF
	Janssens et al[23]	No augmentation of recovery of global LVEF after myocardial infarction
	TOPCARE-AMI[24,25]	Improved LVEF and left ventricular systolic function Reduced infarct size
	REPAIR-AMI[26,27]	Improved LVEF and left ventricular systolic function No myocardial reinfarction Reduction in revascularization procedure Improved myocardial perfusion
	TOPCARE-CHD[28]	Bone marrow derived significantly better than circulating progenitor cells. Improved LVEF and regional contractility
Skeletal myoblasts	MAGIC[29]	No improvement in LVEF Excess ventricular arrhythmias
Cardiosphere-derived cells	CADUCEUS[30]	Reduction in scar mass Improved viable heart mass and regional contractility No improvement in LVEF, left ventricular diastolic and systolic function
Mesenchymal cells	PROMETHEUS	Ongoing
C-kit–positive cardiac stem cells	SCIPIO[31]	Increased LVEF in cell-treated patients

ASTAMI indicates Autologous Stem-Cell Transplantation in Acute Myocardial Infarction; BOOST, BOne marrOw transfer to enhance ST-elevation infarct regeneration; CADUCEUS, CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction; LVEF, left ventricular ejection fraction; MAGIC, Myoblast Autologous Grafting in Ischemic Cardiomyopathy; PROMETHEUS, Prospective Randomized assessment Of MEsenchymal stem cell THErapy in patients Undergoing Surgery; REPAIR-AMI, Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction; CIPIO, Stem Cell Infusion in Patients with Ischemic cardiomyopathy; TOPCARE-AMI, Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction; and TOPCARE-CHD, Transplantation of Progenitor Cells and Recovery of LV [Left Ventricular] Function in Patients with Chronic Ischemic Heart Disease.

Genetic Reprogramming: An Alternative Strategy?

No doubt inspired by demonstrations of genetic reprogramming by Gurdon[7] and Yamanaka[8] which culminated in the 2012 Nobel Prize for Physiology or Medicine, several laboratories have attempted to convert fibroblasts into cardiomyocytes. In a conceptually similar approach to that taken by the Yamanaka group to reprogram cells to pluripotency,[8] recent reports have demonstrated that it is possible to generate iCMs from fibroblasts by overexpressing either transcription factors[9,11] or microRNAs,[10] or both.[12]

Ieda et al[9] observed that overexpression of 3 transcription factors—Gata4, Mef2c, and Tbx5, in either cardiac or dermal murine fibroblasts—could convert them efficiently into a cell type similar to cardiomyocytes. These iCMs showed similar gene expression profiles and epigenetic patterns to true cardiomyocytes, had organized sarcomeric structure, and most convincingly began to spontaneously contract. Further through an elegant series of genetic experiments, they showed that this process is a direct conversion of cell phenotype and does not occur by a preliminary step of dedifferentiation to a progenitor cell type. Gata4, Mef2c, and Tbx5 are key components of a conserved gene regulatory network that control heart morphogenesis, promote cardiac differentiation, and induce the cardiomygenic gene expression program.[50,51] This study also illustrated that a precise combination of transcription factor activity is required for cardiac reprogramming. For example, addition of the cardiac transcription factor, Nkx2-5, dramatically reduced the iCM conversion frequency, despite the fact it collaborates with the 3 iCM reprogramming transcription factors during heart development.[52,53]

Song et al[11] similarly report reprogramming of differentiated mouse adult fibroblasts to iCMs by overexpression of 4 transcription factors. It included the 3 transcription factors (Gata4, Mef2c, and Tbx5) and Hand2, another transcription factor important in cardiac patterning and cardiomyocyte differentiation.[54] Once again, the cells produced by the protocol demonstrated a cardiac-like gene expression profile, began to beat, and had calcium transients similar to true cardiomyocytes. Overexpression of these 4 factors in vivo in the hearts of mice with left anterior descending (coronary artery) ligation-induced myocardial infarction led to conversion of cardiac fibroblasts to cardiomyocyte-like cells and halted the decline in ejection fraction seen in the mice not exposed to the 4 transcription factors.

Jayawardena et al[10] were able to produce a similar reprogramming effect, albeit at lower efficiency, by the application of microRNA mimetics. Fibroblasts were transiently exposed to mimics of 4 microRNAs—miR-1, miR-133, miR-208, and miR-499—reported to regulate cardiac development and differentiation via post-transcriptional repression of a wide range of targets (Table 2). A proportion of the resultant cells expressed mature cardiomyocytes markers, such as cardiac troponin I, myosin heavy chain, L-type calcium channels, and α-actinin, had organized sarcomeres, beat spontaneously, and had oscillatory calcium activity. Furthermore, lentiviral overexpression of the combination of these 4 microRNAs induced in vivo reprogramming of cardiac fibroblasts in a mouse myocardial infarction model.

Finally, Nam et al[12] were able to produce a similar reprogramming effect in human fibroblasts. Three of the same transcription factors as the mouse studies were used, GATA4, Tbx5, and Hand2, together with a fourth transcription factor,

Table 2. Potential Targets of Cardiac Reprogramming miRs and Their Role in Cardiovascular Biology

miRNA	Putative Targets	Relevant Function of Target Gene
miR-1	Hand 2[55]	Promotes ventricular cardiomyocyte proliferation, regulates ventricular morphogenesis and septation
	IRX5[55]	Regulates cardiac conduction via potassium channel Kcnd2
	Dll-1[55]	Promotes cardiac lineage.
	Cdk9[56]	Negative regulation of myocardial differentiation in 2D culture
	MEF2a, calmodulin[55]	Negative regulation of calcium signaling Attenuated cardiomyocyte hypertrophy
	Kir2.1, connexin43[55]	Regulates arrhythmogenic potential
	Hes1[57]	Promotes cardiac gene expression
miR-133	CyclinD2, SRF[55]	Negative regulation of cardiomyocyte and myoblast proliferation Inhibits smooth muscle gene expression
	Cdc42, RhoA, WHSC2[55]	Inhibits cardiac hypertrophy
miR-208	Thrap1[55]	Regulates myofiber gene programs to hypothyroidism Promotes β-MHC expression
	Myostatin[55]	Promotes hypertrophic growth
	GATA4, connexin40[55]	Regulates cardiac conduction system
	Sox6, Purβ, Sp3[55]	Promotes slow myosin expression
	HP-1β[55]	Activates slow myofiber gene expression Inhibits calcium sensor repressor of MEF2 transcription factor
miR-499	Sox6, Purβ[58]	Promotes slow myosin expression via β-MHC expression
	Rod1[59]	Regulates mammalian cell differentiation
	Calcineurin, Drp1[60]	Inhibits cardiomyocyte apoptosis and myocardial infarction

MHC indicates myosin heavy chain.

myocardin, and 2 microRNAs, miR-1 and miR-133. Forced expression of these factors induced expression of cardiac genes, and after 1 to 3 months in culture, some cells developed sarcomere-like structures, calcium cycling, and in a small subset of cells, spontaneous contraction. This does, indeed, support the contention that therapeutic application of reprogramming technology to human cells may be a possibility.

Interestingly, 2 of these groups[9,10] made the observation that cardiac-derived fibroblasts could be reprogrammed to cardiomyocyte-like cells with greater efficiency than tail-tip–derived fibroblasts. Although other cell types may be amenable[61,62] to direct cardiac reprogramming, existing evidence suggests the possibility that cardiac fibroblasts are in some way primed for conversion to iCMs. Fibroblasts are mesenchymal cells with morphological characteristics that include the absence of a basement membrane, an oval nucleus, extensive rough endoplasmic reticulum, and a tendency to form cytoplasmic extensions. Although there is currently no unique marker for cardiac fibroblasts, they express vimentin, fibroblast-specific protein-1, periostin, and collagen receptor, discoidin domain receptor 2.[63]

Cardiac fibroblasts produce interstitial collagen, contributing to the myocardial structure and integrating myocardial tissue layers, supporting heart function.[63] Cardiac fibroblasts are pleomorphic, responding to various mechanical, electric, and chemical stimuli, making them major players in cardiac remodeling under pathological states.[63] This includes transdifferentiation of cardiac fibroblasts into myofibroblasts, which secrete extracellular matrix to promote scar formation and fibrosis.[63] This transdifferentiation of fibroblasts to myofibroblasts involves the calcium channel TRPC6 (transient receptor potential cation channel, subfamily C, member 6).[64] Reprogramming differs from endogenous generation of myofibroblasts because a functional cardiomyocyte is generated. Thus, these resident cardiac fibroblasts may constitute an ideal cell population for in situ cell reprogramming to replace lost cardiomyocytes and abrogate pathological remodeling.

Taken together, these remarkable preclinical studies suggest a potential alternative route toward cardiac regeneration by reprogramming of in situ cardiac fibroblasts into a contractile cell phenotype. Unlike skeletal muscle, which requires only one master controller (MyoD [myogenic differentiation]) to convert other cell types to skeletal muscle,[65] the discovery of a set of transcription factors capable of achieving a similar effect for cardiomyocytes took another 20 years.

Many barriers remain to the progress of this strategy toward clinical use, not the least of which is that lentiviruses cannot be used for human therapy because of the dangers of insertional mutagenesis.[66] Manipulation of cells using microRNAs may be a more suitable strategy for any eventual clinical evaluation of efficacy. Genetic reprogramming would require tissue-specific delivery technology to avoid off target effects of the transforming agents. Even within the heart, it is imperative to determine whether one cardiac subpopulation is a better candidate for reprogramming than the other. For example, it is now apparent that not all cardiac fibroblasts are the same, with atrial fibroblasts having enhanced reactivity and a greater fibrotic response than ventricular fibroblasts.[67] A reprogramming therapy targeted to ventricular fibroblasts might be expected to affect systolic function and avoid potential adverse effects on atrial fibroblasts. Furthermore, there is also no guarantee that cardiomyocytes produced by reprogramming would be electrically integrated with existing cardiomyocytes. The risk of arrhythmia may remain, similar to the risk inherent in cell administration. Nevertheless, these studies raise the exciting possibility that guided reprogramming of resident cells within the mammalian heart may represent a strategy to coax the failing heart to recovery. Although much work remains, genetic reprogramming technology has the potential to provide novel therapeutics for the treatment of heart failure, a clinical syndrome greatly in need of a breakthrough.

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Disclosures

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