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Somatic Cell Reprogramming into Cardiovascular Lineages

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Abstract

Ischemic cardiac disease is the leading cause of death in the developed world. The inability of the adult mammalian heart to adequately repair itself has motivated stem cell researchers to explore various strategies to regenerate cardiomyocytes after myocardial infarction. Over the past century, progressive gains in our knowledge about the cellular mechanisms governing fate determination have led to recent advances in cellular reprogramming. The identification of specific factors capable of inducing pluripotent phenotype in somatic cells as well as factors that can directly reprogram somatic cells into cardiomyocytes suggests the potential for these approaches to translate into clinical therapies in the future. While conceptually appealing, the field of cell lineage reprogramming is in its infancy and further research will be needed to improve the efficiency of the reprogramming process and the fidelity of the reprogrammed cells to their in vivo counterpart.

Introduction

While lower vertebrates such as zebrafish are able to regenerate cardiac tissue after injury,^{1–5} the adult mammalian heart shows very little potential to regenerate and instead undergoes a fibrotic response.^{6,7} Thus, the human heart recovers inefficiently from myocardial infarction where as many as 1 billion cardiomyocytes are lost due to complete coronary vessel occlusion.⁸ Hence, ischemic cardiac disease remains the leading cause of death in developed nations, accounting for over 400,000 deaths in the United States each year.⁹ The only cure for ischemic heart failure is whole organ transplantation, which is limited by the number of donor hearts (approximately 2,000 each year in the US) and complicated by infections and immune rejection. The tremendous burden of ischemic heart disease has motivated the exploration of a number of stem cell-based strategies to treat this devastating disease.

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Conflicts of Interest

The authors declare no conflicts.

Cellular differentiation and lineage programming

The generation of therapeutically important cells like cardiomyocytes using readily available cell types remains a considerable challenge for biologists. Pluripotent embryonic stem cells (ESC) can either self-renew or differentiate in what was long thought to be a unidirectional manner towards increasingly specialized cell types of the three embryonic germ layers. The latter process is often represented by Conrad Waddington's description of an epigenetic landscape of differentiation. In this model, more potent cells sit at the peaks of a landscape before rolling irreversibly downward towards deeper valleys representing more differentiated states as the genome activates and silences fate-specific epigenetic markers. As we currently understand it, there are exceptions to this central dogma that may be exploited for the development of cell-based medical treatments. These technologies have arisen in light of a series of fundamental questions scientists have asked in the last century regarding the processes and the mechanisms of cellular differentiation.

Original hypotheses in the late 1800s advocated that cellular differentiation occurs through permanent losses of hereditary information.¹⁰ However, German embryologists Hans Dreisch and Hans Spemann found that separation of the early blastomeres of recently fertilized animal eggs generates two fully-formed animals.¹¹ These "twinning" experiments challenged the hypothesis that cells permanently lose developmental potential as they become more differentiated. After Avery, MacLeod, and McCarthy demonstrated that nuclear DNA - rather than RNA or protein - was the cellular component responsible for bacterial transformations in the early 1940s,¹² Thomas J. Briggs and Robert W. King successfully pioneered the technique of somatic cell nuclear transfer (SCNT) to determine whether irreversible changes to DNA occur during differentiation.¹³ SCNT is a process by which the nucleus of a somatic cell - a cell that is neither a germ cell nor pluripotent - is transferred into an enucleated activated oocyte. Using the fertilized eggs of *Rana pipiens*, Briggs and King transplanted blastula nuclei into enucleated oocytes and successfully generated swimming larvae.¹⁴ Although this suggested that more differentiated cells retain the genetic material necessary to direct the development of an embryo, experiments using nuclei from later stages of development revealed that as the nuclear donor somatic cell becomes more differentiated, it becomes increasingly difficult to generate clones. Briggs and King concluded that genetic potential must ultimately decline as cells differentiate.

Conflicting hypotheses arose, however, when in 1958 Gurdon and colleagues manipulated the cells of frog species *Xenopus Laevis* to show that transplanting nuclei from mature intestinal cells into enucleated oocytes could generate fully developed clones.¹⁵ The debate as to whether terminally differentiated cells contained the potential to generate fully-formed organisms remained unresolved until fairly recently, when in 1996 Dolly the sheep was cloned by SCNT from mammary epithelial cells.¹⁶ In the past decade, more conclusive answers were provided in studies that cloned mice from the nuclei of definitively differentiated cell types such as adult lymphocytes, which rearrange specific parts of their genomes during differentiation, and post-mitotic neurons.^{17,18}

SCNT experiments established that the genomes of differentiating cells are not irreversibly altered, with the exception of a few types of specialized cells such as lymphocytes, which

alter specific parts of their genomes to perform their immunologic functions. As a result, researchers became more interested in the mechanisms that bring about changes that distinguish cells of one lineage from another, even as they share the same genome. This interest in epigenetics, defined as “the study of stable alterations in gene expression potential that arise during development and cell proliferation,”¹⁹ has steadily gained greater interest from the scientific community over the past 40 years. Epigenetic alterations such as DNA methylation^{20–24} and histone and nucleosome modifications^{25–27} underlie the variegated display of cell lineages seen in nature. SCNT experiments further corroborated the idea that cellular phenotypes could be altered by specific epigenetic changes in the nucleus induced by, in this case, the introduction of an oocyte cytoplasmic environment.²⁸

Although SCNT was critical in establishing a foundational understanding of cell fate, it has been a difficult tool to use. The efficiency of cloning remains very low and most clones exhibit phenotypic and gene expression abnormalities, indicating that SCNT often fails to fully reprogram somatic nuclei.^{29–31} Thus, while SCNT could theoretically be employed to derive pluripotent stem cells from which patient or disease-specific cell types can be generated for scientific investigation or even cell transplantation, these technical obstacles render SCNT an inefficient tool to harness for therapeutic purposes.³²

As SCNT is not well suited for genetic and biochemical studies, a novel approach to studying reprogramming on the molecular level was found in fusion hybrids. After major technical breakthroughs in isolation and culturing strategies for embryonic carcinoma cells and embryonic stem cells,^{33–36} it was demonstrated that the fusion of these pluripotent cells with somatic cells leads to the acquisition of a pluripotent cell phenotype in the somatic cell nucleus.^{37–39} These results corroborated the findings from SCNT studies and further suggested that there are specific cytoplasmic trans-acting factors in pluripotent cell types that can reprogram somatic nuclei.

The hypothesis that specific factors are responsible for nuclear reprogramming was bolstered by experiments that revealed the cell fate-determining role of “master regulatory” genes.⁴⁰ In 1987, Davis et al. identified a single cDNA encoding a gene called MyoD that, when transfected into fibroblasts, could stably convert fibroblasts into skeletal myoblasts.⁴¹ This experiment represented the first documented attempt to deliberately reprogram cells between differentiated lineages *in vitro* by an overexpression of a transcription factor. Soon after, others showed that infection with retroviruses overexpressing MyoD could convert a variety of differentiated cell types into myoblasts.⁴² These successes were followed by work from Xie et al. in 2005 showing that overexpression of myeloid transcription factor C/EBP α could convert primary B and T cells into macrophages.⁴³

Reprogramming of somatic cells to pluripotent stem cells

Prior to 2006, reports of successful reprogramming events in adult mammalian cells were rare and often limited to those that could be accomplished using a single “master” transcription factor. Takahashi and Yamanaka then undertook a novel shotgun approach to combinatorially screen 24 candidate genes that were known to be expressed in ES cells and suspected to play important roles in maintaining pluripotency.⁴⁴ From these, they found that

forced expression of only four genes (i.e. the Yamanaka factors - Oct4, Sox2, c-Myc and Klf4) conferred a pluripotent phenotype in murine fibroblasts. These so-called induced pluripotent stem cells (iPSC) could generate teratomas containing cells of all three germ layers upon subcutaneous transplantation into nude mice.

Shortly after, it was found that the ability of the four Yamanaka factors to reprogram fibroblast into iPSC is conserved in a number of different species, including humans.⁴⁵⁻⁴⁹ Furthermore, a variety of somatic cell types can be reprogrammed into pluripotent stem cells.⁵⁰⁻⁵⁴ However, many of the first attempts to generate iPSC resulted in cells that were only partially reprogrammed as they showed differential gene expression and gene methylation patterns when compared to bona fide ESC. Furthermore, these cells failed to contribute to postnatal chimeras or show germline transmission when injected into developing blastocysts.^{44,55-57}

More recently, investigators have uncovered that a rare number of iPSC lines are truly pluripotent and can generate live born mice by tetraploid complementation.⁵⁸⁻⁶⁰ However, not all iPSC lines are entirely equivalent to ESCs.^{59,61,62} Despite this, the discovery of iPSC has spurred the generation of patient-specific iPSC for disease modeling, drug screening, and therapeutic application.³²

The ability to generate patient-specific cell types could circumvent the difficulties of allogeneic organ transplantation where immune challenges continue to plaque long term outcomes. Furthermore, the genetic etiology of disease could be corrected *ex vivo* using tools of modern genetics.³² For example, genetically-corrected iPSC have been used to treat a mouse model of sickle cell anemia.⁶³ Nevertheless, there remain significant obstacles in applying autologous iPSC to correct diseases of many organ systems.

One obstacle that has been enthusiastically addressed by investigators has been the use of integrating viruses in protocols to create iPSC. These viruses are known to increase the risk for tumors, making iPSC and their derivatives incompatible for patient use. Gene therapy trials in patients with X-linked severe combined immunodeficiency showed that integrating viruses activated proto-oncogene LMO2 and resulted leukemia.⁶⁴ While it may be possible to screen all iPSC for their viral integration sites,⁶⁵ the use of c-Myc, a known oncogene, as one of the four reprogramming factors increases the risks of transgene reactivation.⁶⁶ Many groups have since shown that iPSC can be generated without permanent viral integration using adenoviruses,⁶⁷ plasmids,⁶⁸ modified RNA,⁶⁹ or transgene cassettes expressing the four factors that can be removed via methods like Cre-mediated excision.⁷⁰ Even so, there is a risk for iPSC to acquire other *de novo* genetic mutations as they are manipulated and proliferated *in vitro*; therefore, it may be necessary to sequence the genomes of iPSC clones to ensure that there are no unexpected integrations of viral or exogenous plasmid fragments that might cause spontaneous mutations.⁶⁵

A second obstacle for those attempting to derive specific cell types from ESC or iPSC for direct transplantation is the ability to generate pure populations of desired cell types. Studies of embryonic development have led to the identification of key factors and culture conditions that aid the directed differentiation of ESC or iPSC into different lineages,⁷¹ but

efficient generation of therapeutically useful cells that faithfully reproduce their functions *in vivo* remains challenging.⁷² Differentiation of cardiomyocytes from human ESC or iPSC using embryoid bodies produces heterogeneous cell populations with variable levels of efficiency depending on the iPSC line.⁷³ Modifications to this protocol may increase the efficiency of differentiation,⁷⁴ and a recent report suggests that an alternative monolayer platform can generate greater than 30% cardiomyocytes in some iPSC lines.⁷⁵ Furthermore, the use of small molecule activator and inhibitor of the Wnt pathway has been shown to enhance iPSC differentiation to reliably generate greater than 90% cardiomyocytes from each preparation. Indeed, the ability to efficiently generate and purify populations of usable cardiomyocytes is critical to the progress of iPSC derived cardiac therapies. Remaining issues, such as epigenetic “memory” of the iPSC and *in vitro* culture-induced chromosomal mutation, may pose additional barriers to translation of these cells into human therapy. Thus, source of starting cells for reprogramming may influence the efficiency of iPSC differentiation towards a desired lineage.^{65,76–80}

Third, in addition to the challenge of efficiently generating pure populations of many therapeutically useful cell types, there remain significant concerns that hidden within a heterogeneous population of differentiating cells are a few inclined to divide uncontrollably.^{81–83} Fortunately, methods have been reported that allow for the selective removal of undifferentiated cells; Tang et al. recently used an antibody against SSEA-5 glycan and two other PSC surface markers to cause a loss of teratoma-forming potential in differentiated human ESC cultures.⁸⁴ Further research will improve these strategies to remove undesirable cells thereby increasing the safety of ESC/iPSC –derived cell therapies.

Lastly, for all iPSC-based therapies, the challenges of cellular transplantation remain daunting. Factors that may contribute to the poor engraftment efficiency in the damaged heart include ischemia, anoikis, and inflammation-related processes. Laflamme et al. report, for example, that a cocktail of pro-survival factors may enhance cellular engraftment in the rat heart.⁸⁵ However, the overall engraftment rate remains less than 5% of the starting cell population. Hence, future studies should focus on optimizing the transplantation process (e.g. cell harvesting, storage, and injection) to enhance engraftment and survival in order to bring iPSC and ESC-based therapies closer to the bedside.

Direct Cell Lineage Reprogramming

While improvements in methods and technologies may eventually eliminate the risks of teratoma formation in ESC- or iPSC-based therapies, many of the aforementioned safety obstacles for this technology remain unresolved. Meanwhile, alternative approaches to generating therapeutically valuable cells are being explored. Somatic stem cells and tissues, for example, are attractive cell sources for cell-based therapies as they are readily available in the body and do not have the potential to form teratomas like pluripotent stem cells.

A strategy that has received significant attention is the reprogramming of one somatic cell type to another without passing through a pluripotent state. This approach, termed “transdifferentiation” or “direct reprogramming,” was inspired by the shotgun approach use by Takahashi et al. to generate the first iPSC. Different research groups have screened

combinatorial sets of transcription factors to find those that can induce cells to change from one somatic cell type to another. Zhou et al. first reported the direct reprogramming of adult pancreatic exocrine cells to β -cells *in vivo* by the viral overexpression of three transcription factors, isolated from a candidate pool of 30.⁸⁶

Other successes include the generation of functional neurons,^{87–89} neural progenitors,⁹⁰ blood progenitors,⁹¹ endothelial cells,⁹² and hepatocyte-like cells⁹³ from mouse fibroblasts using a small number of overexpressed transcription factors. Since fibroblasts represent a readily available cell population, it is not surprising that many of the studies described thus far have employed fibroblasts as a starting cell source (Figure 1). As one would expect, the resulting cell types of interest in these early direct reprogramming efforts are often ones that are therapeutically valuable. As such, the direct reprogramming of dopaminergic neurons from both murine and human fibroblast *in vitro* via the overexpression of Mash1, Nurr1, Lmx1a⁸⁹ was likely inspired by the finding that transplantation of ESC-derived dopaminergic neurons improves motor symptoms in a mouse model of Parkinson's disease.^{94,95}

Even among successful reports of direct reprogramming, there remain significant concerns regarding the efficiency of reprogramming and the fidelity of the resulting phenotypes to their gold standards *in vivo*. Since reprogrammed cells may no longer be proliferative, the efficiency of reprogramming must be also high enough for this approach to be considered for therapeutic uses.

Direct reprogramming of fibroblasts into cardiomyocytes

While many groups continue to explore cell therapy-based treatments for cardiac diseases using *in vitro* ESC- or iPSC-derived cardiomyocytes, the efficient deployment of these cardiomyocytes for therapy remains challenging. This has led to recent efforts to directly reprogram somatic cells to cardiomyocytes. One of the first reports of this effort was published by Sheng Ding and colleagues in which they reprogrammed murine fibroblasts into cardiomyocytes using iPSC-generating factors without allowing these cells to pass through a pluripotent state (Figure 1).⁹⁶ After infecting fibroblasts with Oct4, Sox2 and Klf4, cells were maintained in a LIF-free culture condition to prevent the induction or maintenance of a pluripotent cell phenotype. Subsequently, growth factors and small molecules were introduced to redirect partially reprogrammed cells into cardiomyocytes. They showed that this procedure is significantly more efficient and faster than the generation of cardiomyocytes by iPSC *in vitro* differentiation. Interestingly, this method appears to generate mostly atrial cardiomyocytes, which may be therapeutically less useful than ventricular cardiomyocytes.

Another reprogramming method to generate cardiomyocytes from somatic cells has been recently studied in the developing embryo. Takeuchi and Bruneau found that mouse mesoderm can be directed into becoming contracting cardiomyocytes through the overexpression of Gata4, Tbx5, and BAF60c (a cardiac-specific subunit of BAF chromatin remodeling complexes that modulates the transcription of genes).⁹⁷ Given the need for

embryonic tissues as the starting cell source for this reprogramming strategy, it is unclear whether this combination of factors will be useful in adult somatic cell reprogramming.

Following the work of Takeuchi and Bruneau, Ieda et al. examined whether mouse cardiac and tail tip fibroblasts can be directly reprogrammed into cardiomyocytes by virally-mediated overexpression of Gata4, Mef2c and Tbx5 (GMT) (Figure 1).⁹⁸ These authors found that GMT induces murine cardiac and tail tip fibroblasts into becoming cardiomyocyte-like cells that express cardiac troponin T and alpha-myosin heavy chain; the resulting cells could be further triggered to generate spontaneous action potentials *in vitro*. These “induced cardiomyocytes” (iCM) are reportedly adult cardiomyocyte-like in that they lack an expression history of precardiac mesoderm and cardiac progenitor cell markers such as Mesp1 or Isl-1, respectively. Furthermore, fibroblasts infected *in vitro* are reportedly able to transdifferentiate *in vivo* into cardiomyocytes after transplantation into immunocompromised murine hearts. The epigenetic conversion of fibroblasts into cardiomyocyte-like cells was supported by the loss of repressive histone methylations and activation of permissive histone markings in promoter regions of cardiac genes.

Given the difficulty of translating ESC/iPSC-derived cardiomyocytes into therapy where cardiomyocyte enrichment, elimination of partially differentiated cells, and improvements in cell survival after transplantation remain unresolved issues, there is a growing enthusiasm for direct reprogramming as a novel approach to generate cardiomyocytes. This report from Ieda et al. suggests a potential paradigm shift in our current approach to cardiac regenerative therapy as it promises the ability to inject transcription factors to reprogram endogenous cardiac fibroblasts at or near the site of ischemia/infarction.⁹⁹ However, many questions remain as to whether the efficiency of this reprogramming method is sufficient to generate a large number of cardiomyocytes for therapeutic application,¹⁰⁰ particularly when only a small percentage of fibroblasts can be reprogrammed *in vitro* into cardiomyocyte-like cells with low proliferative ability. To address the issue of efficiency, other groups have demonstrated that the addition of other transcription factors to the reprogramming cocktails, such as Myocardin,¹⁰¹ Hand2,¹⁰² or microRNAs¹⁰³ may improve the efficiency of reprogramming (Table I). Moreover, Qian et al. report that *in vivo* reprogramming through direct myocardial injection of GMT factor can achieve greater efficiency of fibroblast conversion into cardiomyocyte-like cells than reprogramming *in vitro*, leading to attenuation of infarct size and improvement in cardiac function.¹⁰⁴

While these studies appear quite promising, questions remain regarding some of the discrepancies noted between the efficiency of reprogramming *in vitro* versus *in vivo*. The efficiency of *in vivo* reprogramming is remarkably higher (10–20% cell conversion) than the efficiency of *in vitro* reprogramming (1–5% or less). The most likely explanation for this difference is that the *in vivo* microenvironment provides soluble or contact factors that enable rapid phenotypic conversion of fibroblasts into cardiomyocytes.¹⁰⁴ In addition, it is possible that the injected virus is able to concentrate itself within the relatively small extracellular compartment in the adult heart to achieve a much greater biological effect locally. Nevertheless, the Cre/LoxP technology used to identify iCM from reprogrammed fibroblast remains fraught with confounding issues (e.g. reactivated Cre expression in the setting of myocardial injury, direct promotion of Cre expression by rare integration of

lentiviruses into existing cardiomyocytes, leaky Cre expression in cardiomyocytes in the absence of viral infection, etc). Confirmation of the increased reprogramming efficiency in vivo by different laboratories using distinct methodologies will be key to sustain the interest in this technology from the cardiovascular research community.

Intriguingly, the factors used to reprogram murine and human fibroblasts appear somewhat distinct (Table II). While GMT factors were not sufficient for the direct reprogramming of human cardiac fibroblasts, the addition of *Mesp1* and *Myocd* successfully generated more cardiac-like cells in vitro.¹⁰⁵ Others have reported that a combination of transcription factors (*Gata4*, *Tbx5*, *Hand2*, *myocardin*, *Mesp1*) and microRNAs (*miR-1*, *miR-133*) among other factors can more effectively reprogram human fibroblasts towards cardiac phenotypes.^{106,107}

As the direct cardiac reprogramming field is relatively new, an in-depth analysis of the iCM phenotypes created by each cocktail of factors has not been published. Thus far, no study has performed a direct side-by-side comparison of the phenotypes of iCM generated from the direct differentiation of iPSC with cardiomyocyte-like cells generated from direct reprogramming, or a bona fide cardiomyocyte from adult heart. While it has been demonstrated that iPSC/ESC can be differentiated into both atrial and ventricular cardiac phenotypes,^{108–111} most studies of direct reprogramming have yet to fully characterize the electrophysiological and contractile parameters of their resultant cell populations. Similarly, iCM from direct reprogramming may also bear different epigenetic modifications than those differentiated from iPSC/ESC⁷⁶ as the former does not pass through a progenitor state, but no study has explored this in detail. Further research on the functional phenotypes and genetic signatures of iCM will elucidate how they compare with iPSC/ESC-derived cardiomyocytes as potential tools for cardiac therapy.

In addition to the need for more detailed understandings of the biology of iCM, considerable improvements in the basic methods of reprogramming must be made before the therapeutic promise of this approach might be realized. First, the use of viral vectors should be minimized to prevent the risk of tumor formation.¹¹² Second, the targeting of transcription factor to fibroblasts and not endothelial or smooth muscle cell should be optimized in order to minimize off-target effects. Finally, the efficiency, speed, and quality of conversion from cardiac fibroblasts to cardiomyocytes should be sufficiently high (e.g. >50% of cells introduced with factors converted to fully mature cardiomyocytes within 5 to 10 days of transduction) for this strategy to be therapeutically relevant in the post-infarct setting to prevent adverse remodeling.

Despite these challenges, the direct reprogramming of endogenous cardiac fibroblasts to iCM after myocardial ischemic injury remains promising as a therapeutic tool because the technique does not require ex vivo manipulation, which has been shown to potentiate genotoxicity,¹¹³ or cellular transplantation, which remains difficult to optimize to achieve sufficient cell survival, and engraftment.^{85,100} With the development of innovative methodologies to safely and efficaciously deliver reprogramming factors, the direct reprogramming strategy may become a viable therapeutic option for cardiac patients in the future.

Conclusion/Future Perspective

The recent successes of cardiomyocyte generation from reprogramming approaches build upon half a century of basic developmental biology research. Early investigators sought to establish that the genetic content of cells do not change throughout differentiation, leading to future studies involving fusion hybrids and somatic cell nuclear transfer. Better understanding of the molecular determinants of genetic expression – from epigenetic states to “master regulator genes” – have opened the doors to the direct reprogramming of somatic cells into therapeutically valuable cell types.

In the future, the application of direct reprogramming strategy may allow a patient with a recent myocardial infarction to undergo a treatment regimen whereby a cocktail of factors is introduced into the scar tissue at the site of infarction. This treatment would aim to reprogram the endogenous fibroblasts into *bona fide* cardiomyocytes, thereby diminishing the scar size and increasing the cardiomyocyte content and improving overall cardiac function.^{102,104}

At the present time, however, numerous challenges of reprogramming such as efficiency, scalability, and quality of the reprogrammed cells must be overcome in order to realize such a therapeutic promise. The availability of a stringent and uniform standard for the characterization of reprogrammed cardiomyocytes will help to guide the generation of fully functional cells.¹¹⁴ Concurrently, considerable progress needs to be made in order to improve the maturation of in vitro iPSC/ESC-derived or reprogrammed cardiomyocytes. With additional efforts, the transplantation of reprogrammed cardiomyocytes or the in situ reprogramming of cardiac fibroblasts directly into new cardiomyocytes may prove feasible for clinical application in the near future.

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Figure 1. Three principal strategies to achieve somatic cell reprogramming and the relative quality of the final reprogrammed cell type

Therapeutically important cells may be generated via somatic cell nuclear transfer (SCNT), *in vitro* differentiation of induced pluripotent stem cells (iPSC), and direct reprogramming of somatic cells without an intermediate pluripotent state.

Table 1

Summary of iCM direct reprogramming studies in murine tissues

	Mouse				
	in vitro			in vivo	
	Embryonic fibroblasts	Cardiac fibroblasts	Tail tip fibroblasts	Cardiac fibroblasts, infected in vitro, transplanted in vivo	Cardiac fibroblasts, direct infection
Efe et al. 2010 [†] Oct4, Sox2, Klf4, c-Myc, JAK inhibitor 1, BMP4	X	X			
Ieda et al. 2010 GMT		X	X	X	
Chen et al. 2012 GMT		X	X	X	
Protze et al. 2012 Mef2c, Tbox 5, Myocd	X	X	X		
Song et al. 2012 [†] GMT, Hand2		X	X		X
Jayawardena et al. 2012 miR-1, miR-133, miR-208, miR-499	X	X	X		X
Qian et al. 2012 [†] GMT + TGFβ					X

[†] Study used retroviruses. Studies used lentiviral vectors unless otherwise indicated.

Table 2

Summary of iCM direct reprogramming studies in human tissues

	Human			
	in vitro			
	Embryonic fibroblasts	Neonatal dermal fibroblasts	Adult dermal fibroblasts	Cardiac fibroblasts
Wada et al. 2013 [†] GMT, Mesp1, Myocd		X		X Adult
Nam et al. 2013 [†] Gata4, Tbx5, Hand2, Myocd, miR-1, MiR-133		X	X	X Adult
Fu et al. 2013 [†] GMT, ESRRG, Mesp1, Myocd, ZFPM2, TGFβ	X Differentiated from hESC	X		X Fetal

[†]Study used retroviruses.[‡]Study used retroviruses and lentiviruses.