UCSF
UC San Francisco Previously Published Works

Title

Recent advances in direct cardiac reprogramming

Permalink

https://escholarship.org/uc/item/9ck5m0xx

Authors

Srivastava, Deepak
Yu, Penghzi

Publication Date

2015-10-01

DOI

10.1016/j.gde.2015.09.004

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

HHS Public Access

Author manuscript

Curr Opin Genet Dev. Author manuscript; available in PMC 2016 October 24.

Published in final edited form as:

Curr Opin Genet Dev. 2015 October ; 34: 77–81. doi:10.1016/j.gde.2015.09.004.

Recent Advances in Direct Cardiac Reprogramming

Deepak Srivastava[1,2,3] and **Penghzi Yu**[1]

[1]Gladstone Institute of Cardiovascular Diseases, San Francisco, CA 94158, USA

[2]Department of Pediatrics, University of California, San Francisco, San Francisco, CA 94158, USA

[3]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94158, USA

Abstract

Human adult cardiomyocytes have limited regenerative capacity resulting in permanent loss of cardiomyocytes in the setting of many forms of heart disease. In an effort to replace lost cells, several groups have reported successful reprogramming of fibroblasts into induced cardiomyocyte-like cells (iCMs) without going through an intermediate progenitor or stem cell stage in murine and human models. This direct cardiac reprogramming approach holds promise as a potential method for regenerative medicine in the future and for dissecting the regulatory control of cell fate determination. Here we review the recent advances in the direct cardiac reprogramming field and the challenges that must be overcome to move this strategy closer to clinical application.

Introduction

Heart disease remains the leading cause of morbidity and mortality in developed countries [1]. Currently, there are no solutions to replenish cardiomyocytes lost to heart injury. The lost cardiomyocytes in the injured region are replaced by scar tissue formed from activated fibroblasts and the extracellular matrix secreted by those fibroblasts [2]. Mammalian hearts do appear to have a small amount of cardiomyocyte turnover over a lifetime [3,4], however it is insufficient for meaningful regeneration.

Although cell-based efforts for regenerative therapy are aggressively being pursued, an alternative approach to regenerate an injured heart is to directly reprogram resident cardiac fibroblasts (CFs) into induced cardiomyocyte-like cells (iCMs) using a cocktail of developmental regulatory proteins that normally guide cardiac fate during cardiogenesis. While this approach has its own limitations and obstacles, it circumvents some of the obstacles of cell-based therapy, including efficient transplantation and integration within the

Correspondence: Deepak Srivastava, M.D., Gladstone Institute of Cardiovascular Disease, 1650 Owens Street, San Francisco, CA 94158, USA. Telephone: 415-734-2716; dsrivastava@gladstone.ucsf.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

area of injured myocardium and creation of mature cardiomoycytes for transplantation. Here, we will discuss advances in direct cardiac reprogramming and consider the challenges and potential of this strategy for regenerative medicine.

Reprogramming of Mouse Fibroblasts into Cardiomyocyte-like Cells

In 2010, our group reported that mouse cardiac and dermal fibroblasts could be converted into cardiomyocyte-like cells in vitro with ectopic expression of three transcription factors: Gata4, Mef2c, and Tbx5 (GMT) [5]. This strategy was inspired by the successes in molecular reprogramming from somatic cells into induced pluripotent stem cells (iPSCs) [6–8] and provided a new potential strategy to regenerate cardiomyocytes. Similar to iPSC reprogramming, a larger population of cells were partially reprogrammed, but full reprogramming to a contractile state occurred with similar frequency as true iPSC colonies [9]. Unlike iPSCs, however, the iCMs quickly exited the cell cycle and did not form colonies [5]. Despite the low percentage of fully reprogrammed cardiomyocytes, genome-wide transcriptome studies of cells activating the α-myosin heavy chain (α-MHC)-reporter revealed that the partially reprogrammed population induced a broad cardiac transcriptional program involving hundreds of genes and also broadly silenced the fibroblast transcriptome. This was an epigenetically stable event and overexpression of the exogenous factors was not necessary after approximately 2 weeks [5].

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 electrical activity noted in ES- or iPS-derived cardiomyocytes. Using a Cre-based strategy, we found that iCMs failed to express Mesp1 or Isl1, markers of early cardiac progenitors, during the process of cardiac reprogramming [5]. This suggested that the reprogramming event represented a direct conversion from one post-natal somatic cell type to another rather than traversing through a progenitor stage. The rapidity of initial conversion and the more mature electrophysiology observed in iCMs is consistent with this interpretation.

In Vivo Cardiac Reprogramming

The initial intention of the *in vitro* reprogramming effort was to ultimately harness the large pool of endogenous CFs as an alternative resource for cardiac regeneration in situ. Accordingly, in 2012, three groups found that in vivo delivery of the GMT transcription factors directly into the heart using a gene therapy approach converted endogenous mouse non-myocytes into iCMs [9–11]. The mice had decreased infarct size and attenuated cardiac dysfunction after coronary ligation and in vivo GMT delivery [10]. Direct reprogramming upon co-administration of Thymosin β4, a 43-amino-acid G-actin monomer-binding protein that can promote angiogenesis, as well as cell survival, proliferation and migration [12,13] was enhanced significantly. Furthermore, it was reported that addition of one more transcription factor, Hand2, with GMT (referred to as GHMT) improved mouse cardiac reprogramming efficiency in vitro, and also resulted in the regeneration of cardiomyocytes in vivo with improved function [9]. In vitro, the GHMT appears to result in a spectrum of ventricular, atrial and conduction cell types [14].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Remarkably, Jayawardena et al. reported in vivo cardiac reprogramming using microRNAs in a mouse model of myocardial infarction [15]. In their pilot in vitro screening, a combination of miR-1, -133, -208, -499 was found to be sufficient to convert cardiac fibroblasts to cardiomyocyte-like cells in vitro and in vivo. This microRNA-mediated conversion could be further enhanced by the addition of JAKI inhibitor, in agreement with the cardiac reprogramming study using iPSC factors by Efe et al [16]. miR-1 appeared to be the most important miRNA, consistent with its essential role in regulating appropriate cardiac sarcomerogenesis and gene expression [17–19]. Recent in vivo data suggests that the combination of miRNAs introduced with a lentivirus after infarct can result in generation of new myocytes and improved cardiac function [20].

In addition to generation of new myocytes, in vivo reprogramming in each study was associated with a significant reduction in fibrosis. It is possible that the newly emerged iCMs secrete certain factors that may inhibit expression of collagen and the activity of matrix metalloproteinase, thus reducing cardiac fibrosis. Furthermore, fibroblasts that were infected by reprogramming factors but failed to reprogram may be intrinsically altered and therefore may have impaired ability to promote fibrosis. It is likely that a combination of these effects ultimately leads to the significant restoration of heart function and decrease in scar after injury.

Enhancing Efficiency of Direct Cardiac Reprogramming

Since the publication of the initial mouse study of in vitro cardiac reprogramming in 2010, several groups have reported methods to improve the efficiency of this approach. Here we discuss the recent results that reported improvements in mouse cardiac reprogramming by 1) altering the combination of reprogramming factors [9,21–25], 2) manipulating signaling pathways [26,27], or 3) optimizing the stoichiometry of the reprogramming factors [28]. Some of the strategies for improved reprogramming efficiency could lead to future breakthroughs in translating this approach and in understanding the underlying biology behind the cell fate transition.

1) Altering the combination of reprogramming factors

The first minimal cardiac reprogramming cocktail, GMT, was established by a reductionist approach similar to those used in mouse and human iPS cell studies [6,8]. While this approach is suitable for finding the minimal set of reprogramming factors, it may not necessarily identify the optimal combination. Furthermore, the master regulatory genes function in self-reinforcing networks and activate one another, suggesting that multiple combinations could lead to similar establishment of the cardiac gene network. Figure 1 summarizes the published approaches for cardiac reprogramming and while there are numerous combinations of factors, GMT is common to most, suggesting that they establish a core regulatory network for the cardiac fate. A few examples are explored below.

- Hirai et al. fused a transactivation domain from MyoD to individual factors in the GHMT cocktail and found that a Mef2c C-terminus fusion with the MyoD transactivation domain plus wild-type Gata4, Hand2 and Tbx5 accelerated cardiac reprogramming, created larger beating clusters from mouse embryonic

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

fibroblasts with a 15-fold greater efficiency than GHMT without the fusion [22]. This is consistent with the observation that reprogramming requires high levels of gene expression and activity to overcome the high barrier of cellular stability inherently present in adult somatic cells [6].

- Protze et al. screened 120 triplet combinations of 10 candidate factors delivered by lentiviral expressing vectors in mouse embryonic fibroblasts (MEFs) with a quantitative PCR panel of five cardiac marker genes (Myh6, Myl2, Actc1, Nkx2.5, Scn5a) as readout. They found the set of Mef2c, Tbx5, and Myocd was the optimal cardiac reprogramming cocktail [23].
- To assess functional success from cardiac reprogramming, the Gearhart laboratory developed a calcium indicator GCaMP reporter driven by human cardiac troponin T (TNNT2) promoter in a lentivirus expression system. With this calcium reporter system, they tested several combinations of cardiac reprogramming factors in mouse embryonic and adult cardiac fibroblasts. They observed that a combination of Hand2, Nkx2.5, Gata4, Mef2c, and Tbx5 (referred to as HNGMT) was the most effective with a 50-fold greater reprogramming efficiency than GMT in cardiac fibroblasts [24].
- Christoforou et al. started from 10 transcription factors (nine human genes and Mesp1 from mouse) delivered by a Tet-On lentivirus expression system in four modules, and found that MYOCD with SRF, or MYOCD, SRF, SMARCD3 and Mesp1 significantly enhanced the cardiac reprogramming effect of human GMT in mouse embryonic fibroblasts [21]. The same report found that valproic acid enhanced cardiac reprogramming by two-fold as measured by Actn2 or Tnnt2 expression.
- Recently, Muraoka et al. reported that overexpression of miR-1 or miR-133 with GMT generated iCMs more efficiently from MEFs [25]. Furthermore, the effect from miR-133 addition is sufficient to shorten the time to reprogramming MEFs into beating iCMs from 30 days to 10 and generates sevenfold more beating iCMs than GMT only. They also demonstrated that miR-133 enhances cardiac reprogramming partially by silencing the fibroblasts signature gene network by suppressing Snai1.

2) Manipulating signaling pathways

Since there is no defined culturing system for maintaining mature cardiomyocytes, cardiac reprogramming can be affected by cell culture conditions that different laboratories use and the batch-to-batch variations in serum products. Much work remains to be done to improve cardiac reprogramming efficiency by stimulating or inhibiting the right signaling pathways.

In 2012, Mathison et al. reported that preconditioning infarcted rat hearts with VEGF delivered by AAV enhanced the efficacy of GMT treatment, resulting in an improvement in ejection fraction after injury [26]. It is not known whether the angiogenesis effect from VEGF improves function as an independent effect, or if VEGF improves function by promoting the generation of new iCMs in vivo.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

With the TNNT2-GCaMP reporter system, the Gearhart group set out to test the effect of signaling pathways on cardiac reprogramming. They reported up to five-fold greater reprogramming efficiency by using a TGFβ signaling inhibitor, SB431542, in addition to the HNGMT cocktail, in MEFs and adult mouse cardiac fibroblasts [27]. They also found that adding TGFβ1 or TGFβ2 to the culture system inhibited cardiac reprogramming. It is not clear whether the improved cardiac reprogramming by TGFβ signaling inhibition is due to suppression of fibroblast gene expression programs, as suggested in the induced pluripotent stem cell field [29].

3) Optimizing the stoichiometry of the reprogramming factors

Most of the published studies in the cardiac reprogramming used various viral vectors expressing different combinations of individual factors. The ratio of expression level from each reprogramming factor in the infected fibroblasts is inconsistent in this setting and could contribute to the significant differences in reported reprogramming efficiency. Wang et al. constructed six polycistronic constructs to include all ordered combinations of Gata4, Mef2c and Tbx5 with identical self-cleaving 2A sequences and showed distinct protein levels of the three transcription factors based on the splicing order [28]. They further demonstrated that relatively higher protein level of Mef2c with modest levels of Gata4 and Tbx5 led to more efficient cardiac reprogramming, and an optimized MGT combination with puromycin selection resulted in over 10-fold increase in beating iCMs. This report convincingly showed that the protein ratio from cardiac reprogramming factors could greatly influence the efficiency and quality of iCMs. More importantly, this study established a single vector platform that provides consistent and reproducible cardiac reprogramming potential, and could serve as a springboard to preclinical large animal studies.

Direct Cardiac Reprogramming in Human Cells

In 2013, three reports demonstrated successful cardiac reprogramming in the human system [30–32]. All three groups found that neither the combination of GMT nor GHMT was sufficient to reprogram human fibroblasts into iCMs. Each group used additional factors to successfully achieve some degree of human cardiac reprogramming. Nam et al. found that the combination of four human transcription factors (GATA4, HAND2, TBX5 and MYOCD) plus two microRNAs (miR-1 and miR-133) activated cardiac marker gene expression from human foreskin, cardiac and dermal fibroblasts in vitro. Although the converted cells displayed some sarcomere structures and calcium transients, few cells showed spontaneous contractility after 11 weeks in culture [30]. Wada et al. showed that adding MESP1 and MYOCD to the original GATA4, MEF2C, and TBX5 combination could reprogram human neonatal and adult cardiac fibroblasts into cardiomyocyte-like cells with calcium oscillations [31]. The human iCMs from the Wada 2013 report did not exhibit spontaneously contractility, but developed action potentials and contracted synchronously when co-cultured with murine cardiomyocytes. Our group found that, in addition to GMT, ESRRG and MESP1 are needed to convert human fibroblasts into a cardiomyocyte-like state, and two more transcription factors, MYOCD and ZFPM2, further enhanced human cardiac reprogramming [32]. The human iCMs from our study were reprogrammed at a level similar to mouse in vitro iCMs as determined by global gene expression analysis. However,

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

we did not observe spontaneous contractility from those human iCMs after 16 weeks in culture. A detailed comparison of the three human cardiac reprogramming studies is available in [33].

These studies provide a foundation for direct cardiac reprogramming in the human system. Unlike in the mouse system, cardiac reprogramming in the human cells requires expression from more factors and longer time, and will likely improve with further study. 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.

Challenges and Future Directions for Cardiac Reprogramming

Recent advances made in mouse and human systems indicate that the cardiac reprogramming efficiency is steadily being advanced by various strategies and might eventually become powerful enough for clinical application and disease modeling studies. One intriguing area worth noting is the qualitative difference observed from iCMs between in vitro and in vivo settings [9,10]. iCMs generated in vivo appeared to be more similar to the endogenous cardiomyocytes than those from in vitro studies, indicating potential beneficial effects of environmental cues like extra-cellular matrix, signaling pathways and mechanical or electrical stimulations. It will be important to increase the efficiency of reprogramming, particularly in vitro, in order to be able to use such cells for disease modeling and ultimately to improve the ability to create new myocytes in vivo. Identification of the signals that improve efficiency in vivo is currently under study in many laboratories, and more unbiased searches such as chemical screening or genome-wide inhibition screens will likely reveal critical barriers to direct cardiac reprogramming.

A fascinating feature of direct cellular reprogramming in most cell types is the progressive, yet rapid, alteration of cellular phenotype in the absence of significant cellular division once the reprogramming process has begun. Genome-wide epigenetic and transcriptional changes occur to establish the necessary landscape for a new cell type without progression through a progenitor state. How this change occurs temporally at the epigenetic level is an important area of study and will likely be revealed in the coming years, providing insight into the regulatory networks that can dictate cellular fate.

Acknowledgements

We thank Bethany Taylor and Gary Howard for their editorial support and all Srivastava lab members for helpful discussion. P.Y. was supported by a CIRM training grant (TG2-01160). D.S. was supported by grants from NHLBI/NIH (U01 HL100405, U01 HL098179, R01 HL057181, P01 HL098707), L.K. Whittier Foundation, William Younger Family Foundation, Eugene Roddenberry Foundation, the California Institute for Regenerative Medicine (CIRM), and by NIH/NCRR grant (C06RR018928) to the Gladstone Institutes.

References

1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, et al. Heart disease and stroke statistics--2012 update: A report from the American Heart Association. Circulation. 2012; 125:e2–e220. [PubMed: 22179539]
2. Cleutjens JP, Blankesteijn WM, Daemen MJ, Smits JF. The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. Cardiovasc. Res. 1999; 44:232–241. [PubMed: 10690298]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, et al. Evidence for cardiomyocyte renewal in humans. Science. 2009; 324:98–102. [PubMed: 19342590]
4. Senyo SE, Steinhauser ML, Pizzimenti CL, Yang VK, Cai L, Wang M, Wu TD, Guerquin-Kern JL, Lechene CP, Lee RT. Mammalian heart renewal by preexisting cardiomyocytes. Nature. 2013; 493:433–436. [PubMed: 23222518]
5. Ieda M, Fu J, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell. 2010; 142:375–386. [PubMed: 20691899] This was the first description of direct reprogramming of fibroblasts to induced cardiomyocyte-like cells using GMT.
6. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126:663–676. [PubMed: 16904174]
7. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131:861–872. [PubMed: 18035408]
8. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318:1917–1920. [PubMed: 18029452]
9. Song K, Nam YJ, Luo X, Qi X, Tan W, Huang GN, Acharya A, Smith CL, Tallquist MD, Neilson EG, et al. Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature. 2012; 485:599–604. [PubMed: 22660318] This paper demonstrated that GMT plus Hand2 could reprogram mouse cardiac fibroblasts into iCMs in vivo after injury, resulting in functional improvement.
10. Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu J, Srivastava D. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature. 2012; 485:593–598. [PubMed: 22522929] This paper demonstrated that GMT could reprogram mouse cardiac fibroblasts into iCMs in vivo after injury, resulting in functional improvement.
11. Inagawa K, Miyamoto K, Yamakawa H, Muraoka N, Sadahiro T, Umei T, Wada R, Katsumata Y, Kaneda R, Nakade K, et al. Induction of cardiomyocyte-like cells in infarct hearts by gene transfer of Gata4, Mef2c, and Tbx5. Circ. Res. 2012; 111:1147–1156. [PubMed: 22931955]
12. Bock-Marquette I, Saxena A, White MD, Dimaio JM, Srivastava D. Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. Nature (article). 2004; 432:466–472.
13. Smart N, Risebro CA, Melville AA, Moses K, Schwartz RJ, Chien KR, Riley PR. Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization. Nature. 2007; 445:177–182. [PubMed: 17108969]
14. Nam YJ, Lubczyk C, Bhakta M, Zang T, Fernandez-Perez A, McAnally J, Bassel-Duby R, Olson EN, Munshi NV. Induction of diverse cardiac cell types by reprogramming fibroblasts with cardiac transcription factors. Development. 2014; 141:4267–4278. [PubMed: 25344074]
15. Jayawardena TM, Egemnazarov B, Finch EA, Zhang L, Payne JA, Pandya K, Zhang Z, Rosenberg P, Mirotsou M, Dzau VJ. MicroRNA-mediated in vitro and in vivo direct reprogramming of cardiac fibroblasts to cardiomyocytes. Circ. Res. 2012; 110:1465–1473. [PubMed: 22539765] This paper demonstrated a direct cardiac reprogramming using a cocktail of microRNAs.
16. Efe JA, Hilcove S, Kim J, Zhou H, Ouyang K, Wang G, Chen J, Ding S. Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. Nat. Cell Biol. 2011; 13:215–222. [PubMed: 21278734]
17. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific mircroRNA that targets *Hand2* during cardiogenesis. Nature. 2005; 436:214–220. [PubMed: 15951802]
18. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell. 2007; 129:303–317. [PubMed: 17397913]
19. Heidersbach A, Saxby C, Carver-Moore K, Huang Y, Ang Y, de Jong PJ, Ivey KN, Srivastava D. microRNA-1 regulates sarcomere formation and suppresses smooth muscle gene expression in the mammalian heart. eLife. 2013; 2:e01323. [PubMed: 24252873]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

20. Jayawardena TM, Finch EA, Zhang L, Zhang H, Hodgkinson CP, Pratt RE, Rosenberg PB, Mirotsou M, Dzau VJ. MicroRNA induced cardiac reprogramming in vivo: evidence for mature cardiac myocytes and improved cardiac function. Circ. Res. 2015; 116:418–424. [PubMed: 25351576]
21. Christoforou N, Chellappan M, Adler AF, Kirkton RD, Wu T, Addis RC, Bursac N, Leong KW. Transcription factors MYOCD, SRF, Mesp1 and SMARCD3 enhance the cardio-inducing effect of GATA4, TBX5, and MEF2C during direct cellular reprogramming. PLoS One. 2013; 8:e63577. [PubMed: 23704920]
22. Hirai H, Katoku-Kikyo N, Keirstead SA, Kikyo N. Accelerated direct reprogramming of fibroblasts into cardiomyocyte-like cells with the MyoD transactivation domain. Cardiovasc. Res. 2013; 100:105–113. [PubMed: 23794713]
23. Protze S, Khattak S, Poulet C, Lindemann D, Tanaka EM, Ravens U. A new approach to transcription factor screening for reprogramming of fibroblasts to cardiomyocyte-like cells. J. Mol. Cell. Cardiol. 2012; 53:323–332. [PubMed: 22575762]
24. Addis RC, Ifkovits JL, Pinto F, Kellam LD, Esteso P, Rentschler S, Christoforou N, Epstein JA, Gearhart JD. Optimization of direct fibroblast reprogramming to cardiomyocytes using calcium activity as a functional measure of success. J. Mol. Cell. Cardiol. 2013; 60:97–106. [PubMed: 23591016]
25. Muraoka N, Yamakawa H, Miyamoto K, Sadahiro T, Umei T, Isomi M, Nakashima H, Akiyama M, Wada R, Inagawa K, et al. MiR-133 promotes cardiac reprogramming by directly repressing Snai1 and silencing fibroblast signatures. EMBO J. 2014
26. Mathison M, Gersch RP, Nasser A, Lilo S, Korman M, Fourman M, Hackett N, Shroyer K, Yang J, Ma Y, et al. In vivo cardiac cellular reprogramming efficacy is enhanced by angiogenic preconditioning of the infarcted myocardium with vascular endothelial growth factor. J. Am. Heart Assoc. 2012; 1:e005652. [PubMed: 23316332]
27. Ifkovits JL, Addis RC, Epstein JA, Gearhart JD. Inhibition of TGFbeta signaling increases direct conversion of fibroblasts to induced cardiomyocytes. PLoS One. 2014; 9:e89678. [PubMed: 24586958]
28. Wang L, Liu Z, Yin C, Asfour H, Chen O, Li Y, Bursac N, Liu J, Qian L. Stoichiometry of Gata4, Mef2c, and Tbx5 influences the efficiency and quality of induced cardiac myocyte reprogramming. Circ. Res. 2015; 116:237–244. [PubMed: 25416133]
29. Maherali N, Hochedlinger K. Tgfbeta signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. Curr. Biol. 2009; 19:1718–1723. [PubMed: 19765992]
30. Nam YJ, Song K, Luo X, Daniel E, Lambeth K, West K, Hill JA, DiMaio JM, Baker LA, Bassel-Duby R, et al. Reprogramming of human fibroblasts toward a cardiac fate. Proc. Natl. Acad. Sci. USA. 2013; 110:5588–5593. [PubMed: 23487791] This paper reported the ability to reprogram human fibroblasts into iCMs.
31. Wada R, Muraoka N, Inagawa K, Yamakawa H, Miyamoto K, Sadahiro T, Umei T, Kaneda R, Suzuki T, Kamiya K, et al. Induction of human cardiomyocyte-like cells from fibroblasts by defined factors. Proc. Natl. Acad. Sci. USA. 2013; 110:12667–12672. [PubMed: 23861494] This paper reported the ability to reprogram human fibroblasts into iCMs.
32. Fu J, Stone NR, Liu L, Spencer CI, Qian L, Hayashi Y, Delgado-Olguin P, Ding S, Bruneau BG, Srivastava D. Direct reprogramming of human fibroblasts toward the cardiomyocyte lineage. Stem Cell Reports. 2013; 1:235–247. [PubMed: 24319660] This paper reported the ability to reprogram human fibroblasts into iCMs.
33. Fu JD, Srivastava D. Direct reprogramming of fibroblasts into cardiomyocytes for cardiac regenerative medicine. Circ. J. 2015; 79:245–254. [PubMed: 25744738]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

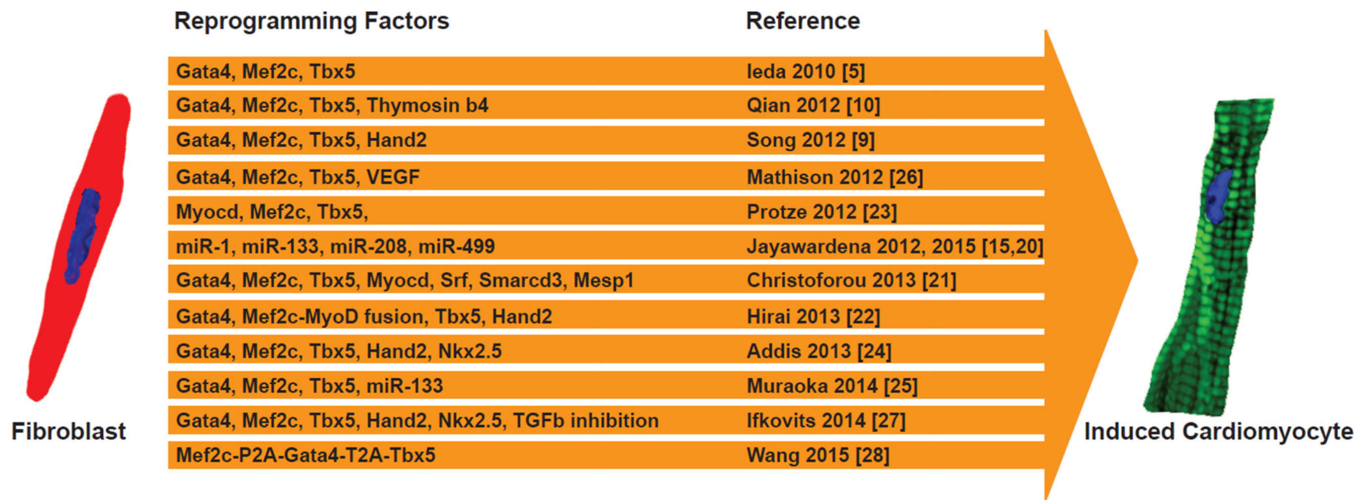

Figure 1.

Different combinations of reprogramming factors used to convert fibroblasts into cardiomyocyte-like cells in mouse.