View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Biotechnology

Cardiomyocyte generation from somatic sources — current status and future directions

Michael G Monaghan[1], Monika Holeiter[1], Shannon L Layland[1,2] and Katja Schenke-Layland[1,2,3]

Transdifferentiation of one cell type to another has garnered significant research efforts in recent years. As cardiomyocyte loss following myocardial infarction becomes debilitating for cardiac patients, the option of an autologous source of cardiomyocytes not derived from multi/pluripotent stem cell sources is an attractive option. Such direct programming has been clearly realized with the use of transcription factors, microRNAs and more recently small molecule delivery to enhance epigenetic modifications, all albeit with low efficiencies *in vitro*. In this review, we aim to present a brief overview of the current *in vitro* and *in vivo* transdifferentiation strategies in the generation of cardiomyocytes from somatic sources. The interdisciplinary fields of tissue, cell, material and regenerative engineering offer many opportunities to synergistically achieve directly programmed cardiac tissue *in vitro* and enhance transdifferentiation *in vivo*. This review aims to present a concise outlook on this topic with these fields in mind.

Addresses
[1] Department of Women's Health, Research Institute for Women's Health, Eberhard Karls University, Tübingen, Germany
[2] Department of Cell and Tissue Engineering, Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB), Stuttgart, Germany
[3] Department of Medicine/Cardiology, Cardiovascular Research Laboratories, University of California, Los Angeles, CA, USA

Corresponding author: Schenke-Layland, Katja (katja.schenke-layland@med.uni-tuebingen.de)

Current Opinion in Biotechnology 2016, **40**:49–55

This review comes from a themed issue on **Tissue, cell and pathway engineering**

Edited by **April Kloxin** and **Kyongbum Lee**

For a complete overview see the Issue and the Editorial

Available online 3rd March 2016

http://dx.doi.org/10.1016/j.copbio.2016.02.014

0958-1669/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Cardiovascular disease is one of the world's leading causes of mortality. Myocardial infarction (MI) is the death of heart tissue due to ischaemia, typically caused by the blockage of blood flow to an area in the heart. Resident cardiomyocytes have a very limited capacity to proliferate in the adult heart, resulting in the lack of heart regeneration post-MI [1]. To date, the most efficient therapy for heart failure is whole organ transplantation, which is limited by donor hearts availability, compromised by immunosuppressant therapy and an invasive procedure not suitable for all patients.

Cell therapies have been of interest to researchers due to their variety of cell sources, the ability to scale-up *in vitro* and their potential to improve the regeneration of tissue. This has evolved from research with autologous stem cell sources — bone marrow-derived stem cells, adipose tissue-derived stem and progenitor cells, all of which have reached clinical trials [2]. Recent approaches to induce a pluripotent state in various adult somatic cells, termed induced-pluripotent stem cells (iPSCs), has resulted in exciting work towards clinical therapy and disease modelling [3••]; however, returning cells to a pluripotent state raises concerns of teratoma formation and possible unwarranted differentiation [4]. Prompted by the advent of iPSCs [3••], the concept of a direct transition from one determined cell type into another (transdifferentiation) by overexpressing transcription factors, microRNAs (miRs) and/or delivering small molecules has emerged [5••,6••,7••]. Almost 30 years ago, myogenic features in fibroblasts were being driven by introducing the expression of the muscle-specific transcription factor MyoD [8]. This direct conversion was achieved by epigenetic suppression of the fibroblast phenotype and progressive activation of the target cell via cDNA transfections. Transdifferentiation has since been reported for cell types such as pancreatic beta cells [9], neurons [10], hepatocyte-like cells [11], and haematopoietic progenitor cells [12]. Inducing functional cardiomyocytes (iCMs) directly from fibroblasts was first reported with murine cells in 2010 [5••]. Since then, substantial efforts have been applied to increase transdifferentiation efficiencies [13••]. Gradually, the incorporation of additional stimuli such as dynamic cultures, mechanical, topographical and extracellular matrix (ECM) cues, along with other lessons learned from stem cell and iPSC differentiation is slowly impacting the direct reprogramming protocols with increased efficiencies. In this review, we aim to discuss the important developments in the transdifferentiation of fibroblasts to iCMs *in vitro* and *in vivo* with the goal of highlighting developments in the field of tissue engineering and biomaterials design that could realize exciting accomplishments in this field.

Driving transdifferentiation using cardiac transcription factors

Typically, cardiac fibroblasts maintain the structural and paracrine sustenance of adjacent cardiomyocytes. However, activation of these fibroblasts occurs after MI and subsequently they migrate to the site of injury and synthesize fibrotic ECM as a compensatory structure for the compromised myocardium [1]. The abundance of cardiac fibroblasts in the injured heart intuitively highlights them as a target for reprogramming, whereby they could offer as a source for cardiac regeneration. Cardiac fibroblasts and cardiomyocytes, in theory, should share many epigenetic features as they both derive from a common progenitor cell population [14]. The significance of the originating cell type and its natural environment has been reported in myogenic [15] and pancreatic beta cell reprogramming [9]. In both cases, somatic cells originating from different germ layers to that of the envisaged cell type failed to yield successful transdifferentiation.

The most documented and the first factors to derive iCMs are the transcription factors Gata4, Mef2c and Tbx5 (GMT). Since their initial reporting [5••], many reprogramming cocktails have been tested, most of them virally delivered and based on the original combination of GMT but with additional factors (Mesp1, Hand1, Hand2, Nkx2.5, myocardin (Myocd), Smarcd3 or SRF) to improve reprogramming efficiencies [16••,17–19] (see Table 1 for an overview). G, M, and T are the prevailing regulators at the peak of the cardiac gene regulatory networks and their expression during normal development follows a delicate pattern [20••]. It is reported that GMT alone is inefficient to produce functional iCMs but results in a partially reprogrammed phenotype expressing transcripts such as cardiac TroponinT but not alpha myosin heavy chain (α-MHC) [21•]. Combining Myocd with Tbx5 and Mef2c to treat neonatal cardiac fibroblasts has resulted in a 2.5% yield of α-MHC-expressing cells 14 days post-transduction (GMT alone achieved 2.2%); however, complete transdifferentiation in the form of beating cells after four weeks was not obtained [22].

It is also reported that a fine balance of the GMT factors is required to accomplish more efficient transdifferentiation [20••,23]. Essentially, a high Mef2c protein level and lower expression level of Gata4 and Tbx5 transpired to be key in yielding iCMs in fibroblasts transduced by a polycistronic vector [20••]. Stoichiometry of the factors has also been found to have an effect through non-viral mRNA delivery [23]. Such a sensitive equilibrium may be one reason why GMT has yielded poor efficiency in other researchers' investigations. Repression of Snai1 has been implicated as an enhancer of GMT transdifferentiation as Snai1 is capable of inducing mesenchymal behaviour and fibrogenesis during development and disease. Knocking down Snai1-expression with siRNA during GMT transduction of MEFs significantly increased the reprogramming efficiency compared to GMT alone [24]. In contrast, overexpressing Snai1 during transdifferentiation inhibited cardiac gene expression and spontaneous beating. Other researchers have noted a fivefold improvement of iCM induction has been achieved via inhibition of TGF-β using SB431542 with transfection of GMT + Hand2 + Nkx2.5 [25]. TGF-β acts as an activator of Snai1. Therefore both studies establish that the repression of Snai1 is important to stop the maintenance of the fibroblast phenotype. Additionally, a more recent study found that although GMT and Hand2 transdifferentiated fibroblasts into beating cells expressing cardiac markers (5%), genes associated with fibrosis were also upregulated in the first week of culture [26••]. On the basis of the hypothesis that fibrotic signalling was hindering transdifferentiation, small molecules to silence TGF-β and Rho associated kinase signalling yielded an efficiency of 60% functional cardiomyocytes from mouse embryonic fibroblasts [26••].

microRNA mediated transdifferentiation

The role of miRs and the disruption of their endogenous levels and cell-specific functions following MI are well reported [27]. The regulatory role of miRs in the suppression of mRNA translation plays an important role in cell fate decisions, which can have a knock-on/off effect on the presence of transcription factors and other stimulatory factors. Jayawardena *et al.* were the first who identified a cocktail of miRs (miR-1, -133, -208, -499) that seemed to preferably transdifferentiate fibroblasts into iCMs [6••]. Within this study; cardiac protein expression, rhythmic calcium oscillations and beating clusters were observed in about 1–2% of the cell population [6••]. Notably, the introductory method of the miRs in this study (non-viral delivery of mature miR mimics) necessitated a single transient transfection.

Muraoka *et al.* investigated the effect of miR-1, -133,-208, and -499 on mouse embryonic fibroblasts (MEFs) isolated from α-MHC promoter-driven eGFP transgenic mice in generating iCMs [24]. This study was not successful in generating iCMs using this defined cocktail of miRs. However, combining GMT viral delivery with just miR-133 (non-viral mature miR mimic) resulted in significantly enhanced transdifferentiation efficiencies in murine and human fibroblasts [24]. When investigating the cardiomyocyte subtype they observed mostly iCMs of an atrial phenotype. Interestingly, the study detected beating events in GMT+ miR-133 transduced MEFs as early as day 10 post-induction; whereas cells treated with GMT alone did not exhibit beating cells until four weeks post-induction.

Another approach in converting fibroblasts to iCMs is the combination of transiently overexpressing factors generally recognized for iPSC generation, with culture conditions and factors specific to cardiac differentiation, but

obviating a pluripotent state. Efe *et al.* retrovirally transduced MEFs with Oct4, Sox2, Klf4 (OSK) and cultured under defined conditions (LIF-free cardiomyogenic media) using small molecules and growth factors [28•] and induced spontaneously contracting patches of cardiac cells. This study found that small molecule inhibition of JAK-STAT (Janus kinase-signal transducer and activator of transcription) during the initial nine day period and supplementation of BMP4 from day nine gave more beating cells. However, regardless of the culture time, the expression of late stage markers (Mlc-2a) suggested that the generated iCMs were of an atrial subtype. As early as 11 days after transduction, spontaneous contractions were observed and many colonies were beating by day 15. The authors speculated that pluripotency reprogramming factors (especially Oct4) initially remove the cell's identity but epigenetic mechanisms, and soluble factors in a staged protocol of differentiation media are then capable of inducing the desired cell type. More recently, this group demonstrated combining Oct4 [29] with a small molecule cocktail consisting of SB431542 (ALK4/5/7 inhibitor), CHIR99021 (GSK3 inhibitor), parnate (LSD1/KDM1 inhibitor), and forskolin (adenylyl cyclase activator) collectively known as SCPF [29], was sufficient to wipe the fibroblast epigenetic memory, thus enabling improved cell transdifferentiation with cardiomyogenic signals (small molecules and growth factors). In this case, BMP4 was added from day 6 after transduction to induce a cardiomyocyte phenotype. The group observed contracting clusters from day 20 and generated 99 ± 17 beating foci on day 30 after 1×10^4 MEFs were initially plated. Most of the derived cells indicated a ventricular subtype with hardly any displaying atrial or nodal features.

Chemically achieved transdifferentiation

Suppression of the starting cell epigenetic signature is paramount to overcoming one major molecular roadblock for successful transdifferentiation; namely the shutdown of the fibroblast program, before an adoption of the desired cell fate becomes possible. Cells not only undergo transcriptional changes but also exhibit epigenetic changes in DNA methylation and histone modifications [30,31], and it are primarily these changes that convert the epigenetic pattern of somatic cells to an embryonic stem cell-like state. Several small molecules that block and inhibit enzymes involved in epigenetic modifications, including histone methylation or demethylation, can increase the efficiency of transdifferentiation and can sometimes functionally replace ectopic expression of certain transcription factors. Routinely, G9a-mediated H3K9 methylation is necessary for heterochromatinization and silencing of key pluripotency genes, such as Oct4 and Rex1 during early embryogenesis [32]. DNA methyltransferase inhibitor, 5-azacytidine, or histone deacetylase inhibitors (suberoylanilide hydroxamic acid, trichostatin A and valproic acid) improved reprogramming efficiency after transduction of the four iPSC transcription factors in MEFs [33]. A cell's epigenetic memory can be essentially erased by treating established iPSCs with 5-azacytidine and trichostatin A [33]. The use of small molecule compounds in cell transdifferentiation, which could be better accepted for clinical translation, has recently been highlighted with the complete generation of iPSCs and neural progenitor cells via small molecules [34–36]. More recently, transdifferentiating MEFs into cardiomyocytes (sometimes beating) using chemically defined cocktails has been achieved with a transition via a cardiac progenitor cell stage but not that of a pluripotent stage [7••]. Yet still, the induction efficiencies of iCMs using this, and other methods *in vitro* remain disappointingly low.

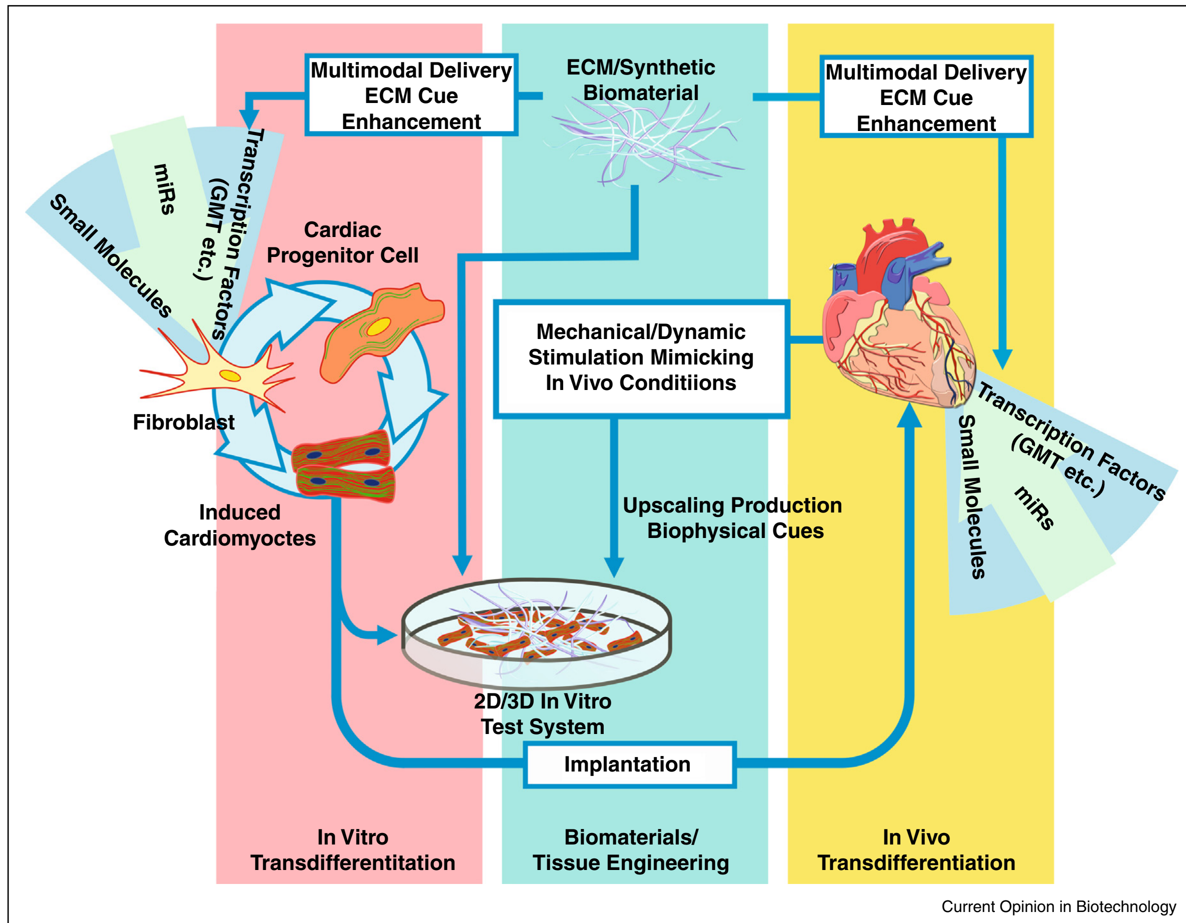
In vivo efforts

Interestingly, *in vivo* approaches of direct cardiac reprogramming applied after experimental MI in mice obtain higher efficiencies than *in vitro* approaches. Considering that the fibrotic scar is primarily composed of ECM-producing fibroblasts, this is indeed promising. Qian *et al.* [37••] and Song *et al.* [16••] have both used genetic lineage tracing to ascertain that in mouse infarcted hearts, transdifferentiation of non-myocytes into functional iCMs occurred. Both studies document improved functional recovery and reduced fibrotic scar tissue. Since then, other improvements to *in vivo* GMT transdifferentiation have been made with respect to the delivery vector [38,39] and preconditioning the myocardium with angiogenic factors [40,41]. miR-based transdifferentiation *in vivo* has also been reported by Jayawardena *et al.* whereby their initial study determined that 1% of the iCMs were of a fibroblast origin [6••] and a more recent study of the therapeutic effect of this treatment found progressive improvement in cardiac function. These conversion rates *in vivo* (1–35%) are encouraging; however, to generate disease-in-a-dish models and *in vitro* iCM yields suitable for transplantation, increased *in vitro* efficiencies are required to achieve large-scale cultures.

The influence of ECM signalling

Many strategies have potential regarding transdifferentiation to generate iCMs *in vitro* from somatic sources and the direct reprogramming of resident cells *in vivo* (Figure 1). The ECM serves as an important component of all tissues, and its composition and mechanical properties play significant roles in the self-renewal or differentiation of cells. ECM composition and signalling in stem cell niches promotes the self-renewal of stem or progenitor cells and this knowledge has been utilized early on in embryonic stem cell culture for ESC maintenance *in vitro* using MEFs secreting ECM [42], ECM-based substrates such as Matrigel® [43], specific ECM proteins such as laminins, collagen type I, or vitronectin [44–46]. ECM proteins have also been utilized to guide stem cell differentiation to somatic cell types, including cardiomyocytes [47–50]. For instance, collagen type IV has been

Figure 1



Direct reprogramming of fibroblasts to a functional cardiomyocyte with or without a progenitor cell intermediate has been widely reported with increasing efficiencies using transcription factors, miRs and small molecules. The evolution of these protocols will benefit greatly by the use of cardio-stimulatory environments with biomaterials, extracellular matrices and dynamic cultures based on lessons learned *in vivo*, which could yield significant efficiencies suitable for implantation. Additionally, using delivery vehicles of transdifferentiation factors that are based on biomaterial and extracellular matrices, which are favourable towards cardiomyogenesis could further improve direct reprogramming *in vivo*.

shown to increase the differentiation of mouse embryonic stem cells into cardiac progenitor cells (CPCs) while fibronectin can enhance CPC differentiation to cardiomyocytes [51]. Additionally, to reprogram somatic cells at least partially to multipotent cells, the use of embryonic stem cell extracts [52] or animal oocyte extracts [53,54] has been described. Zhang *et al.* induced multipotency in fibroblasts by extracellular delivery of the ECM component fibromodulin [55]. Interestingly, the multipotent cells differentiated into derivatives of all three germ layers including cardiomyocytes, skeletal myocytes, neurons, pancreatic lineage cells, osteoblasts, and adipocytes *in vitro* while omitting the risk of teratoma formation *in vivo* [55]. More delivery approaches become available when considering ECM-enhanced iCM generation as the ECM can also serve as a depot for growth factors, transcription factors and nucleic acid vectors (viruses and plasmid constructs) for gene therapy [56]. However, one such factor alone is not enough and therefore payloads that can achieve sustained and programmed release of many molecules at the same, or at staged time intervals are paramount to the correct transdifferentiation of cells *in vivo* [57,58].

Incorporation of biomechanical cues

Research focused on the interplay between physical and developmental cues has demonstrated that mechanical forces generated by cells or tissues are crucial for the control of embryological development, morphogenesis and tissue patterning [59]. The importance of the mechanical properties of a cell's or tissue's microenvironment has been recognized by many in the field of tissue engineering [51,60]. This has resulted in the design of elaborate systems to mimic a native environment with defined mechanical cues of surface rigidity, stretch and strain. Some of these cues exist already *in vivo*, which

could be a strong justification as to why transdifferentiation is more successful *in vivo*. Ruan *et al.* have recently shown that cyclic mechanical stress in 3D *in vitro* cultures of ESC and hiPSC-derived CPCs favoured cardiac differentiation and promoted cardiomyocyte structural and functional maturation [61•]. It is difficult to recapitulate small molecule interventions *in vivo*, which suggests other epigenetic occurrences present in the myocardium. Again, such influences *in vivo* could be ECM signalling. Recently it was shown that topography plays an instrumental role in the epigenetic state of the cell whereby the study of Morez *et al.* cultured adult heart-derived progenitor cells on microgrooves (10 μm wide, 3 μm deep) to enhance histone acetylation and cardiomyocyte differentiation [62•]. The growing range of functional biomaterials that can release drugs, proteins, growth factors and ECM components, or that display an improved mechanical functionality, is currently the focus of tissue engineering and regenerative medicine [63•]. A temporally and spatially controlled release of bioactive molecules from such functional biomaterials can be achieved through the combination of different mechanisms, like diffusion-based release, biomaterial-degradation, or cell-triggered release [64].

Conclusion

The generation of functional cardiac tissue *in vitro* by transdifferentiating somatic cell sources can only be truly realized and up-scaled by combining lessons learned from cardiomyocyte derivation from iPSCs or stem cells, whereby ECM biophysical cues and dynamic cultures have yielded more mature iCMs with higher efficiencies. This would enable the generation of patient-specific drug testing systems and personalized engineering of cardiac tissue *in vitro*.

Acknowledgements

The authors would like to thank Daniel Alejandro Carvajal Berrio (University Hospital Tübingen) for creating Figure 1. This work was financially supported by the European Union (AMCARE, 604531, FP7-NMP-2013-SME-7 to KS-L.; Marie Curie IEF, 331430 to MGM); Fraunhofer-Gesellschaft Internal programs (FFE to SLL), the Ministry of Science, Research and the Arts of Baden-Württemberg (33-729.55-3/214), and the Deutsche Forschungsgemeinschaft (SCHE 701/10-1) (all to KS-L).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.copbio.2016.02.014.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. van den Borne SWM, Diez J, Blankesteijn WM, Verjans J, Hofstra L, Narula J: **Myocardial remodeling after infarction: the role of myofibroblasts**. *Nat Rev Cardiol* 2010, **7**:30-37.

2. George JC: **Stem cell therapy in acute myocardial infarction: a review of clinical trials**. *Transl Res* 2010, **155**:10-19.

3. •• Takahashi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors**. *Cell* 2006, **126**:663-676.
This landmark report documents the first time that fibroblasts were induced to become stem cells (iPSCs) by introduction of four factors; Oct3/4, Sox2, c-Myc, and Klf4, known since as the Yamanaka Factors.

4. Gutierrez-Aranda I, Ramos-Mejia V, Bueno C, Munoz-Lopez M, Real PJ, Mácia A, Sanchez L, Ligero G, Garcia-Parez JL, Menendez P: **Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embryonic stem cells regardless the site of injection**. *Stem Cells* 2010, **28**:1568-1570.

5. •• Ieda M, Fu JD, 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.
The first report of generating induced cardiomyocytes with the generation of beating cells in rare cases. iCMs were identified via α-MHC-GFP and TropT expression. GMT was narrowed down from 14 factors. Mouse cardiac fibroblasts and tail tip fibroblasts generated 4–6% and 2.5% of transdifferentiation respectively.

6. •• 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.
The first iCMs to be generated using miRs. miR-1, -133, -208, -499, and JI1 (an inhibitor of JAK-STAT — Janus kinase-signal transducer and activator of transcription). *In vitro*, 13.4–27.9% of transdifferentiation was achieved from mouse cardiac fibroblasts with spontaneous beating observed after 10 days (1%). When applied *in vivo* following myocardial infarction in a mouse model, 1% of iCMs were detected derived from resident fibroblasts.

7. •• Fu Y, Huang C, Xu X, Gu H, Ye Y, Jiang C, Qiu Z, Xie X: **Direct reprogramming of mouse fibroblasts into cardiomyocytes with chemical cocktails**. *Cell Res* 2015, **25**:1013-1024.
Full chemical transdifferentiation of mouse embryonic fibroblasts to induced cardiomyocytes is reported in this paper. CRFVPTZ (C, CHIR99021; R, RepSox; F, Forskolin; V, VPA; P, Parnate; T, TTNPB; and Z, DZnep). The authors applied a two-step culture period with defined factors and found beating cells as early as 6–8 days. Induced cardiomyoctes were generated from both mouse embryonic fibroblasts and mouse neonatal tail tip fibroblasts.

8. Davis RL, Weintraub H, Lassar AB: **Expression of a single transfected cDNA converts fibroblasts to myoblasts**. *Cell* 1987, **51**:987-1000.

9. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA: **In vivo reprogramming of adult pancreatic exocrine cells to beta-cells**. *Nature* 2008, **455**:627-632.

10. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M: **Direct conversion of fibroblasts to functional neurons by defined factors**. *Nature* 2010, **463**:1035-1041.

11. Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L: **Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors**. *Nature* 2011, **475**:386-389.

12. Batta K, Florkowska M, Kouskoff V, Lacaud G: **Direct reprogramming of murine fibroblasts to hematopoietic progenitor cells**. *Cell Rep* 2014, **9**:1871-1884.

13. •• Doppler SA, Deutsch MA, Lange R, Krane M: **Direct reprogramming — the future of cardiac regeneration?** *Int J Mol Sci* 2015, **16**:17368-17393.
An excellent, comprehensive review with detail to reprogramming for cardiac regeneration.

14. van Wijk B, van den Hoff M: **Epicardium and myocardium originate from a common cardiogenic precursor pool**. *Trends Cardiovasc Med* 2010, **20**:1-7.

15. Weintraub H, Tapscott SJ, Davis RL, Thayer MJ, Adam MA, Lassar AB, Miller AD: **Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced**

expression of MyoD. *Proc Natl Acad Sci U S A* 1989, **86**: 5434-5438.

16. •• Song K, Nam Y-J, 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.

Song and colleagues reported that their retroviral delivery of GMT with the addition of Hand2 into the ischaemic myocardium of mice, converted resident cardiac fibroblasts into functional iCMs (between 2% and 6%). This study also reported improved functional recovery and reduced fibrosis.

17. 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.

18. 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.

19. 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.

20. •• 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.

The stoichiometry of the factors GMT was shown here to be especially important for both αMHC-GFP and TropT expression in induced cardiomyocytes. Higher Mef2c levels gave higher expressions in induced cardiomyocytes generated from mouse cardiac fibroblasts (10% αMHC-GFP expression).

21. • Chen JX, Krane M, Deutsch M-A, Wang L, Rav-Acha M, Gregoire S, Engels MC, Rajarajan K, Karra R, Abel ED *et al.*: **Inefficient reprogramming of fibroblasts into cardiomyocytes using Gata4 Mef2c, and Tbx5**. *Circ Res* 2012, **111**:50-55.

A very interesting paper whereby GMT alone were found to be inefficient to induce cardiomyocytes and no cardiac markers or CPC stage was achieved using MCFs or TTFs.

22. 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.

23. Lee K, Yu P, Lingampalli N, Kim HJ, Tang R, Murthy N: **Peptide-enhanced mRNA transfection in cultured mouse cardiac fibroblasts and direct reprogramming towards cardiomyocyte-like cells**. *Int J Nanomed* 2015, **10**:1841-1854.

24. 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, **33**:1565-1581.

25. 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.

26. •• Zhao Y, Londono P, Cao Y, Sharpe EJ, Proenza C, O'Rourke R, Jones KL, Jeong MY, Walker LA, Buttrick PM *et al.*: **High-efficiency reprogramming of fibroblasts into cardiomyocytes requires suppression of pro-fibrotic signalling**. *Nat Commun* 2015, **6**.

On the basis of the hypothesis that fibrotic signalling was hindering transdifferentiation, small molecules to silence TGF-β and Rho associated kinase signalling yielded an efficiency of 60% functional cardiomyocytes from mouse embryonic fibroblasts.

27. Monaghan M, Greiser U, Wall JG, O'Brien T, Pandit A: **Interference: an alteRNAtive therapy following acute myocardial infarction**. *Trends Pharmacol Sci* 2012, **33**:635-645.

28. • 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.

This study activated the pluripotency of tail tip fibroblasts through delivery of the factors Oct4, Sox2, Klf4 and c-Myc. Defined culture conditions were applied in order to direct towards a cardiomyogenic fate rather than a state of pluripotency using small molecules cytokines. Beating clusters of cells were evident at day 18 and 39% of the cell population had TroponinT expression at day 18.

29. Wang H, Cao N, Spencer CI, Nie B, Ma T, Xu T, Zhang Y, Wang X, Srivastava D, Ding S: **Small molecules enable cardiac reprogramming of mouse fibroblasts with a single factor, Oct4**. *Cell Rep* 2014, **6**:951-960.

30. Hawkins RD, Hon GC, Lee LK, Ngo Q, Lister R, Pelizzola M, Edsall LE, Kuan S, Luu Y, Klugman S *et al.*: **Distinct epigenomic landscapes of pluripotent and lineage-committed human cells**. *Cell Stem Cell* 2010, **6**:479-491.

31. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R *et al.*: **Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution**. *Cell Stem Cell* 2007, **1**:55-70.

32. Feldman N, Gerson A, Fang J, Li E, Zhang Y, Shinkai Y, Cedar H, Bergman Y: **G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis**. *Nat Cell Biol* 2006, **8**:188-194.

33. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA: **Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds**. *Nat Biotechnol* 2008, **26**:795-797.

34. Long Y, Wang M, Gu H, Xie X: **Bromodeoxyuridine promotes full-chemical induction of mouse pluripotent stem cells**. *Cell Res* 2015, **25**:1171-1174.

35. Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, Zhao T, Ye J, Yang W, Liu K *et al.*: **Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds**. *Science* 2013, **341**:651-654.

36. Cheng L, Hu W, Qiu B, Zhao J, Yu Y, Guan W, Wang M, Yang W, Pei G: **Generation of neural progenitor cells by chemical cocktails and hypoxia**. *Cell Res* 2014, **24**:665-679.

37. •• Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway S.J., Fu J-D, Srivastava D: **In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes**. *Nature* 2012, **485**:593-598.

In this study the authors retrovirally delivered GMT to the infarcted myocardium in a mouse model. They reported that 35% of cardiomyocytes in the border/infarct zone were iCMs derived from resident cardiac fibroblasts which was evaluated using lineage tracing. The treated animals also had an improved functional recovery and reduced fibrosis. This report, with other *in vivo* reports improved transdifferentiation *in vivo* compared to *in vitro* reports.

38. 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.

39. Mathison M, Singh VP, Gersch RP, Ramirez MO, Cooney A, Kaminsky SM, Chiuchiolo MJ, Nasser A, Yang J, Crystal RG *et al.*: **"Triplet" polycistronic vectors encoding Gata4 Mef2c, and Tbx5 enhances postinfarct ventricular functional improvement compared with singlet vectors**. *J Thor Cardiovasc Surg* 2014, **148** 1656–1664.e1652.

40. Mathison MP, Gersch R, 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**.

41. Srivastava D, Ieda M, Fu J, Qian L: **Cardiac repair with thymosin β4 and cardiac reprogramming factors**. *Ann N Y Acad Sci* 2012, **1270**:66-72.

42. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A: **Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro**. *Nat Biotechnol* 2000, **18**:399-404.

43. Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, Carpenter MK: **Feeder-free growth of undifferentiated human embryonic stem cells**. *Nat Biotechnol* 2001, **19**:971-974.

44. Jones MB, Chu CH, Pendleton JC, Betenbaugh MJ, Shiloach J, Baljinnyam B, Rubin JS, Shamblott MJ: 1: **Proliferation and pluripotency of human embryonic stem cells maintained on type I collagen**. *Stem Cells Dev* 2010, **19**:1923-1935.

45. Prowse AB, Doran MR, Cooper-White JJ, Chong F, Munro TP, Fitzpatrick J, Chung TL, Haylock DN, Gray PP, Wolvetang EJ: **Long term culture of human embryonic stem cells on recombinant vitronectin in ascorbate free media**. *Biomaterials* 2010, **31**:8281-8288.

46. Rodin S, Domogatskaya A, Strom S, Hansson EM, Chien KR, Inzunza J, Hovatta O, Tryggvason K: **Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511**. *Nat Biotechnol* 2010, **28**:611-615.

47. Zhang J, Klos M, Wilson GF, Herman AM, Lian X, Raval KK, Barron MR, Hou L, Soerens AG, Yu J *et al.*: **Extracellular matrix promotes highly efficient cardiac differentiation of human pluripotent stem cells: the matrix sandwich method**. *Circ Res* 2012, **111**:1125-1136.

48. Schenke-Layland K, Angelis E, Rhodes KE, Heydarkhan-Hagvall S, Mikkola HK, Maclellan WR: **Collagen IV induces trophoectoderm differentiation of mouse embryonic stem cells**. *Stem Cells* 2007, **25**:1529-1538.

49. Schenke-Layland K, Rhodes KE, Angelis E, Butylkova Y, Heydarkhan-Hagvall S, Gekas C, Zhang R, Goldhaber JI, Mikkola HK, Plath K *et al.*: **Reprogrammed mouse fibroblasts differentiate into cells of the cardiovascular and hematopoietic lineages**. *Stem Cells* 2008, **26**:1537-1546.

50. Xue JX, Gong YY, Zhou GD, Liu W, Cao Y, Zhang WJ: **Chondrogenic differentiation of bone marrow-derived mesenchymal stem cells induced by acellular cartilage sheets**. *Biomaterials* 2012, **33**:5832-5840.

51. Schenke-Layland K, Nsair A, Van Handel B, Angelis E, Gluck JM, Votteler M, Goldhaber JI, Mikkola HK, Kahn M, MacLellan WR: **Recapitulation of the embryonic cardiovascular progenitor cell niche**. *Biomaterials* 2011, **32**:2748-2756.

52. Taranger CK, Noer A, Sorensen AL, Hakelien AM, Boquest AC, Collas P: **Induction of dedifferentiation, genomewide transcriptional programming, and epigenetic reprogramming by extracts of carcinoma and embryonic stem cells**. *Mol Biol Cell* 2005, **16**:5719-5735.

53. Hansis C, Barreto G, Maltry N, Niehrs C: **Nuclear reprogramming of human somatic cells by xenopus egg extract requires BRG1**. *Curr Biol* 2004, **14**:1475-1480.

54. Zhu XQ, Pan XH, Wang W, Chen Q, Pang RQ, Cai XM, Hoffman AR, Hu JF: **Transient in vitro epigenetic reprogramming of skin fibroblasts into multipotent cells**. *Biomaterials* 2010, **31**:2779-2787.

55. Zheng Z, Jian J, Zhang X, Zara JN, Yin W, Chiang M, Liu Y, Wang J, Pang S, Ting K *et al.*: **Reprogramming of human fibroblasts into multipotent cells with a single ECM proteoglycan, fibromodulin**. *Biomaterials* 2012, **33**: 5821-5831.

56. Monaghan M, Browne S, Schenke-Layland K, Pandit A: **A collagen-based scaffold delivering exogenous microrna-29B to modulate extracellular matrix remodeling**. *Mol Ther* 2014, **22**:786-796.

57. Browne S, Monaghan MG, Brauchle E, Berrio DC, Chantepie S, Papy-Garcia D, Schenke-Layland K, Pandit A: **Modulation of inflammation and angiogenesis and changes in ECM GAG-activity via dual delivery of nucleic acids**. *Biomaterials* 2015, **69**:133-147.

58. Dash BC, Thomas D, Monaghan M, Carroll O, Chen X, Woodhouse K, O'Brien T, Pandit A: **An injectable elastin-based gene delivery platform for dose-dependent modulation of angiogenesis and inflammation for critical limb ischemia**. *Biomaterials* 2015, **65**:126-139.

59. Mammoto T, Ingber DE: **Mechanical control of tissue and organ development**. *Development* 2010, **137**:1407-1420.

60. Hanjaya-Putra D, Wong KT, Hirotsu K, Khetan S, Burdick JA, Gerecht S: **Spatial control of cell-mediated degradation to regulate vasculogenesis and angiogenesis in hyaluronan hydrogels**. *Biomaterials* 2012, **33**:6123-6131.

61. • Ruan J-L, Tulloch NL, Saiget M, Paige SL, Razumova MV, Regnier M, Tung KC, Keller G, Pabon L, Reinecke H *et al.*: **Mechanical stress promotes maturation of human myocardium from pluripotent stem cell-derived progenitors**. *Stem Cells* 2015, **33**:2148-2157.

Study demonstrating that 3D culture of embryonic stem cell derived cardiac progenitor cells promotes maturation of cardiomyocytes. 2D culture, on the other hand gave rise to more smooth muscle cell maturation.

62. • Morez C, Noseda M, Paiva MA, Belian E, Schneider MD, Stevens MM: **Enhanced efficiency of genetic programming toward cardiomyocyte creation through topographical cues**. *Biomaterials* 2015, **70**:94-104.

Study reporting the culture of adult heart-derived progenitor cells on microgroves enhances histone H3 acetylation, thereby mimicking the effect of chemical compounds, to achieve cariomyogenic differentiation.

63. • Hinderer S, Brauchle E, Schenke-Layland K: **Generation and assessment of functional biomaterial scaffolds for applications in cardiovascular tissue engineering and regenerative medicine**. *Adv Healthc Mater* 2015.

A comprehensive up-to-date review of materials tested in cardiac applications.

64. Chandra P, Lee SJ: 1: **Synthetic extracellular microenvironment for modulating stem cell behaviors**. *Biomark Insights* 2015, **10**:105-116.