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**

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

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