Cell

Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

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DOI 10.1016/j.cell.2010.07.002

SUMMARY

The reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs) raises the possibility that a somatic cell could be reprogrammed to an alternative differentiated fate without first becoming a stem/progenitor cell. A large pool of fibroblasts exists in the postnatal heart, yet no single "master regulator" of direct cardiac reprogramming has been identified. Here, we report that a combination of three developmental transcription factors (i.e., Gata4, Mef2c, and Tbx5) rapidly and efficiently reprogrammed postnatal cardiac or dermal fibroblasts directly into differentiated cardiomyocyte-like cells. Induced cardiomyocytes expressed cardiac-specific markers, had a global gene expression profile similar to cardiomyocytes, and contracted spontaneously. Fibroblasts transplanted into mouse hearts one day after transduction of the three factors also differentiated into cardiomyocyte-like cells. We believe these findings demonstrate that functional cardiomyocytes can be directly reprogrammed from differentiated somatic cells by defined factors. Reprogramming of endogenous or explanted fibroblasts might provide a source of cardiomyocytes for regenerative approaches.

INTRODUCTION

Heart disease is a leading cause of adult and childhood mortality. The underlying pathology is typically loss of cardiomyocytes that leads to heart failure or improper development of cardiomyocytes during embryogenesis that leads to congenital heart malformations. Because postnatal cardiomyocytes have little or no regenerative capacity, current therapeutic approaches are limited. Embryonic stem cells possess clear cardiogenic potential, but efficiency of cardiac differentiation, risk of tumor formation, and issues of cellular rejection must be overcome (Ivey and Srivastava, 2006; Laflamme et al., 2007; Nussbaum et al., 2007; van Laake et al., 2008). The ability to reprogram fibroblasts into induced pluripotent stem cells (iPSCs) with four defined factors might address some of these issues by providing an alternative source of embryonic-like stem cells (Takahashi and Yamanaka, 2006). However, generating sufficient iPSC-derived cardiomyocytes that are pure and mature and that can be delivered safely remains challenging (Zhang et al., 2009).

The human heart is composed of cardiomyocytes, vascular cells, and cardiac fibroblasts. In fact, cardiac fibroblasts comprise over 50% of all the cells in the heart (Baudino et al., 2006; Camelliti et al., 2005; Snider et al., 2009). Cardiac fibroblasts are fully differentiated somatic cells that provide support structure, secrete signals, and contribute to scar formation upon cardiac damage (Ieda et al., 2009). Fibroblasts arise from an extracardiac source of cells known as the proepicardium, and do not normally have cardiogenic potential (Snider et al., 2009). The large population of endogenous cardiac fibroblasts is a potential source of cardiomyocytes for regenerative therapy if it were possible to directly reprogram the resident fibroblasts into beating cardiomyocytes. Unfortunately, although embryonic mesoderm can be induced to differentiate into cardiomyocytes (Takeuchi and Bruneau, 2009), efforts to accomplish this in somatic cells have thus far been unsuccessful, and to our knowledge, no "master regulator" of cardiac differentiation, like MyoD for skeletal muscle (Davis et al., 1987), has been identified to date.

The generation of iPSCs suggests that a specific combination of defined factors, rather than a single factor, could epigenetically alter the global gene expression of a cell and allow greater plasticity of cell type than previously appreciated. Consistent

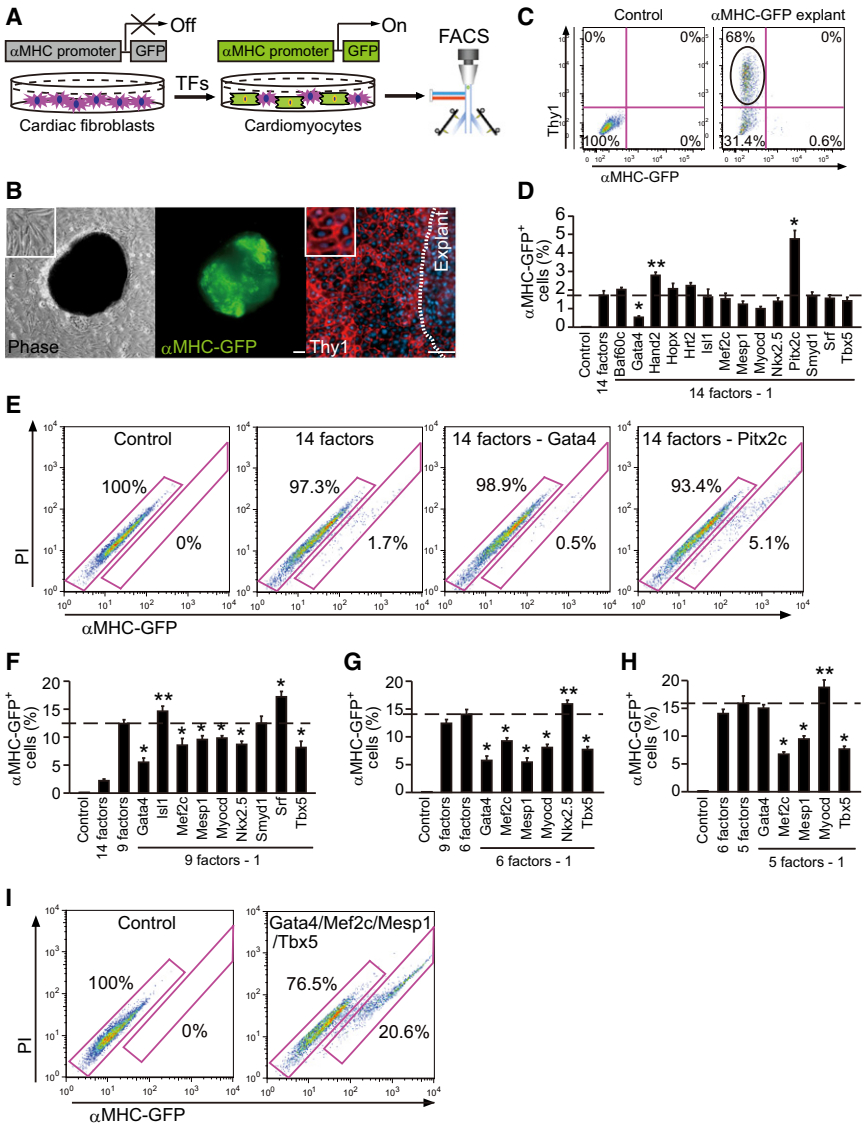

Figure 1. Screening for Cardiomyocyte-Inducing Factors

(A) Schematic representation of the strategy to test candidate cardiomyocyte-inducing factors.

(B) Morphology and characterization of fibroblast-like cells migrating from αMHC-GFP heart explants. Phase contrast (left), GFP (middle), and Thy-1 immunostaining (right). Insets are high-magnification views. See also Figure S1.

(C) Thy-1$^+$/GFP$^-$ cells were FACS sorted from explant cultures for reprogramming.

(D) Summary of FACS analyses for α-MHC-GFP$^+$ cells. Effect on GFP$^+$ cell induction with 14 factors or the removal of individual factors from the pool of 14 factors (n = 3). Removal of Baf60c, Hand2, Hopx, Hrt2, or Pitx2c did not decrease the percent of GFP$^+$ cells and were excluded for further analyses. See also Figure S2.

(E) FACS plots for analyses of GFP$^+$ cells. GFP$^+$ cells were analyzed 1 week after 14 factor transduction. The number of GFP$^+$ cells were reduced by removal of Gata4, but increased by removal of Pitx2c from 14 factors.

(F–H) Effect on GFP$^+$ cell induction of the removal of individual factors from the pool of 9 (F), 6 (G), or 5 (H) factors (n = 3). Factors that did not decrease efficiency upon removal were excluded from further study.

(I) GFP$^+$ (20%) cells were induced from fibroblasts by the combination of four factors, Gata4, Mef2c, Mesp1, and Tbx5. Representative data are shown in each panel. PI, propidium iodine. All data are presented as means ± SD. *$p < 0.01$; **$p < 0.05$ versus relevant control. Scale bars represent 100 μm. See also Figures S1 and S2.

with this, the bHLH transcription factor, Neurogenin 3, in combination with Pdx1 and Mafa, can efficiently reprogram pancreatic exocrine cells into functional β cells in vivo, although the exocrine cells were known to have some potential to become islet cells in vitro and share a common parent cell with islet cells (Baeyens et al., 2005; Zhou et al., 2008). A combination of three factors, Ascl1, Brn2, and Myt1l, converts dermal fibroblasts to functional neurons (Vierbuchen et al., 2010), although the degree of global reprogramming of the neurons is unknown.

In this study, we examined whether key developmental cardiac regulators could reprogram cardiac fibroblasts into cardiomyocytes. We found that out of a total of 14 factors, a specific combination of three transcription factors, Gata4, Mef2c, and Tbx5, was sufficient to generate functional beating cardiomyocytes directly from mouse postnatal cardiac or dermal fibroblasts and that the induced cardiomyocytes (iCMs) were globally reprogrammed to adopt a cardiomyocyte-like gene expression profile.

RESULTS

Screening for Cardiomyocyte-Inducing Factors

We developed an assay system in which the induction of mature cardiomyocytes from fibroblasts could be analyzed quantitatively by reporter-based fluorescence-activated cell sorting (FACS) (Figure 1A). To accomplish this, we generated αMHC promoter-driven EGFP-IRES-puromycin transgenic mice (αMHC-GFP), in which only mature cardiomyocytes expressed the green fluorescent protein (GFP) (Gulick et al., 1991). We confirmed that only cardiomyocytes, but not other cell types such as cardiac fibroblasts, expressed GFP in the transgenic mouse hearts and in primary cultured neonatal mouse cardiac cells (Figure S1 available online).

To have enough cardiac fibroblasts for FACS screening, we obtained GFP$^-$ cardiac fibroblasts from neonatal αMHC-GFP hearts by explant culture. Fibroblast-like cells migrated from the explants after 2 days and were confluent after 1 week. The migrating cells did not express GFP, but expressed Thy1 and vimentin, markers of cardiac fibroblasts (Figure 1B and data not shown) (Hudon-David et al., 2007; Ieda et al., 2009). To avoid contamination of cardiomyocytes, we filtered the cells by cell