Cardiomyocyte cell cycle activation improves cardiac function after myocardial infarction

Rutger J. Hassink1*, Kishore B. Pasumarthi2,3, Hidehiro Nakajima2,3, Michael Rubart2,3, Mark H. Soopaa2,3, Aart Brutel de la Rivière4, Pieter A. Doevendans1, and Loren J. Field2,3

1Department of Cardiology, University Medical Center, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands; 2The Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202-5225, USA; 3Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN 46202-5225, USA; and 4Department of Cardio-thoracic Surgery, University Medical Center, Heidelberglaan 100, 3584, CX, Utrecht, The Netherlands

Received 23 October 2007; revised 22 November 2007; accepted 4 December 2007; online publish-ahead-of-print 12 December 2007

Time for primary review: 20 days

KEYWORDS
Cardiomyocyte cell cycle activation;
Cardiac regeneration;
Myocardial infarction;
Cardiac function

Aims Cardiomyocyte loss is a major contributor to the decreased cardiac function observed in diseased hearts. Previous studies have shown that cardiomyocyte-restricted cyclin D2 expression resulted in sustained cell cycle activity following myocardial injury in transgenic (MHC-cycD2) mice. Here, we investigated the effects of this cell cycle activation on cardiac function following myocardial infarction (MI).

Methods and results MI was induced in transgenic and non-transgenic mice by left coronary artery occlusion. At 7, 60, and 180 days after MI, left ventricular pressure–volume measurements were recorded and histological analysis was performed. MI had a similar adverse effect on cardiac function in transgenic and non-transgenic mice at 7 days post-injury. No improvement in cardiac function was observed in non-transgenic mice at 60 and 180 days post-MI. In contrast, the transgenic animals exhibited a progressive and marked increase in cardiac function at subsequent time points. Improved cardiac function in the transgenic mice at 60 and 180 days post-MI correlated positively with the presence of newly formed myocardial tissue which was not apparent at 7 days post-MI. Intracellular calcium transient imaging indicated that cardiomyocytes present in the newly formed myocardium participated in a functional syncytium with the remote myocardium.

Conclusion These findings indicate that cardiomyocyte cell cycle activation leads to improvement of cardiac function and morphology following MI and may represent an important clinical strategy to promote myocardial regeneration.

1. Introduction
Cardiomyocyte death is a common end-point in many forms of cardiovascular disease. It is generally agreed that cardiomyocytes in the adult mammalian heart exhibit some capacity to re-enter the cell cycle.1 In addition, recent studies suggested that adult-derived stem cells might contribute to cardiomyocyte renewal in injured hearts.2,3 However, it is very clear that these intrinsic processes are of insufficient magnitude to restore cardiac function after myocardial infarction (MI). A number of approaches have been explored to increase cardiomyocyte number in injured hearts, with the hope that this would promote functional recovery. These include direct transplantation of cardiomyocytes or myogenic stem cells,4,5 treatment with cytokines to mobilize endogenous stem cells,6 and cardiomyocyte cell cycle activation.7,8

Commitment to a new round of cell division requires transit through the restriction point.9,10 Restriction point transit is regulated by the activity of the cyclin-dependent kinases CDK2 and CDK4, and their obligate co-factors, the D-type cyclins. Cycl/CDK activity in turn is positively regulated by growth factors and negatively regulated by members of the CIP and KIP CDK inhibitor families. Previous studies generated transgenic mice expressing D-type cyclins under the transcriptional regulation of the alpha-cardiac myosin heavy chain (MHC) promoter in an effort to promote cardiomyocyte cell cycle progression.11,12 Targeted expression of cyclin D1, D2, or D3 resulted in increased base line levels of cardiomyocyte DNA synthesis in the adult myocardium. Cardiomyocyte DNA synthesis persisted following myocardial injury in the cyclin D2 transgenic mice (designated MHC-cycD2 mice), but not in the cyclin D1 and D3 mice. Sustained cell cycle activity in MHC-cycD2 mice was accompanied by an increase in cardiomyocyte cell number and concomitant reduction of infarct size.12

* Corresponding author: Tel: +31 73 6992000; fax: +31 73 6992763. E-mail address: rjhassink@orange.nl; rjhassink@wanadoo.nl

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Although a previous study demonstrated a progressive improvement in cardiac architecture post-MI, it was not clear whether this was associated with the appearance of functional cardiomyocytes and a concomitant improvement in cardiac function. In this study, the impact of cardiomyocyte cell cycle activity on cardiac function was examined following myocardial injury. MHC-cycD2 mice and their non-transgenic siblings were subjected to permanent coronary artery occlusion. Cell cycle activity resulted in the accumulation of newly formed myocardium in the MHC-cycD2 transgenic mice. Intracellular calcium transient imaging indicated that the newly formed myocardium was functionally coupled to the remote myocardium. Moreover, intraventricular pressure-volume measurements revealed a positive correlation between the presence of newly formed myocardium and improved cardiac function in the MHC-cycD2 transgenic mice. In contrast, no improvement in cardiac structure or function was observed in the non-transgenic siblings. These findings support the notion that cardiomyocyte cell cycle activation can restore function in injured hearts.

2. Methods

2.1 Transgenic mice

The generation of the MHC-cycD2 transgenic line was described previously. These animals expressed a mouse cyclin D2 cDNA under the transcriptional regulation of the mouse α-cardiac MHC promoter. Mice were maintained in a DBA/2J inbred background. Male mice were used for all studies. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

2.2 Myocardial infarction model

MI was induced by ligation of the left coronary artery as described. Briefly, the animals were intubated and ventilated with 2% isoflurane and supplemental oxygen. Via left thoracotomy, the left coronary artery was ligated at the inferior border of the left auricle. The intercostal space and the skin incision were then closed with interrupted sutures, the endotracheal tube was removed, and the animal placed on a 37°C heating pad (Cole Parmer, Vernon Hills, IL, USA) under a 100% oxygen cover for 24 h post-surgery. Sham-operated animals underwent the same procedure without ligation of the coronary artery.

2.3 Cardiac function analysis

Pressure-volume measurements were obtained as described before. At 7, 60, or 180 days post-MI or sham-operation, mice were anesthetized with 2% isoflurane supplemented with 100% oxygen, intubated with an endotracheal tube, and ventilated (Mini-vent 845; Hugo Sachs Elektronik, March-Hugstetten, Germany) at 125 cycles/min and a tidal volume of 6–7 μL/g. Mice were placed in supine position under a dissection microscope and connected to a feedback heating-lamp via a rectal temperature sensor for the maintenance of stable body temperature at 37°C. A pre-calibrated four-electrode 1.4 F pressure-volume (P-V) catheter (Model SPR-839; Millar Instruments, Houston, TX, USA) was inserted into the right common carotid artery and advanced into the left ventricle (LV). The catheter was connected to a pressure-conductance unit (Sigma SA; CD Leycom, Zoetermeer, The Netherlands). The continuous pressure and volume signals were monitored in real time and digitized at a sample rate of 500 s⁻¹, using specialized software (Conduct NT; CD Leycom, Zoetermeer, The Netherlands) on a notebook computer. The display of the online pressure–volume signals allowed for optimal positioning of the catheter within the LV.

After a short period of stabilization, LV pressure–volume loops were recorded at baseline, and the signals were acquired three times for 5 s with the ventilator stopped. This yielded a total of 120–150 cardiac cycles from which the following parameters were determined using specialized Cirlab analysis software (Leiden University Medical Center, Leiden, The Netherlands): heart rate (HR), LV end-systolic pressure (Pes), LV end-diastolic pressure (Pd), LV change in positive and negative pressures (dP/dtmax and dP/dtmin, respectively), and LV isovolumic relaxation time constant (Tau). After the steady-state measurements, pressure-volume relations were measured three times by transiently occluding the inferior vena cava. The end-systolic pressure-volume relation (ESPVR; end-systolic elastance: Ees) and the slope of the dP/dtmax with respect to volume (dP/dtmax/EDV) were derived from the acquired cardiac cycles.

2.4 Histology and immunohistochemistry

After functional analysis, hearts were harvested, perfusion fixed at physiological pressure with formalin, and embedded in paraffin using standard protocols. Coronal sections were sampled from apex to base at 1.0 mm intervals and stained with Azan (Sigma) according to the manufacturer’s protocol. Digital photographs were taken and infarct size was calculated using the following formula: [length of coronal infarct perimeter (epicardial + endocardial)/total LV coronary perimeter (epicardial + endocardial)] × 100. Other sections were stained for connexin43 (connexin43 rabbit polyclonal antibody, Zymed, South San Francisco, CA, USA) using standard techniques.

2.5 Calcium transient imaging of peri-infarct myocardium

Hearts were prepared for two-photon molecular excitation (TPME) laser scanning microscopy and perfused with oxygenated normal Tyrode’s solution containing 50 μmol/L cytochalasin D as described previously. Images were recorded with a Bio-Rad MRC 1024 laser scanning microscope modified for TPME. Illumination for two-photon excitation was provided by a mode-locked Ti:Sapphire laser (Spectraphysics, Mountain View, CA, USA); the excitation wavelength was 810 nm. Hearts were imaged through a Nikon 60×1.2 numerical aperture water-immersion lens with a working distance of 200 μm. Images were collected at a resolution of 0.43 μm per pixel along the xy-plane. For full-frame mode analyses (512 × 512 pixels), hearts were scanned at 1.46 and 0.73 frames per second on horizontal (x, y) planes and the resulting images digitized at eight-bit resolution and stored directly on the hard disk. For line-scan mode analyses, hearts were scanned repetitively along a line spanning at least two juxtaposed cardiomyocytes (scan speed was 110 μm/ms at a rate of 32 Hz). Line-scan images were then constructed by stacking all lines vertically. Post-acquisition analysis was performed using MetaMorph software version 4.6r (Universal Imaging Corporation, Downingtown, PA, USA).

2.6 Statistical analysis

All data are presented as mean ± SEM. Between-group comparisons were analysed by unpaired t-test. Significance was assumed at P < 0.05.

3. Results

3.1 Infarct size

Adult non-transgenic mice and their MHC-cycD2 transgenic siblings were subjected to experimental MI via permanent coronary artery occlusion. There was no difference in
mortality between transgenic and non-transgenic animals. Hearts were harvested at 7, 60, and 180 days post-MI and fixed under physiological pressure. Coronal sections sampled at 1 mm intervals from the apex to the base of hearts were used to calculate infarct size using standardized assays.12,17 There was no significant difference in infarct size in hearts harvested from the non-transgenic mice at any time point (infarct size was 52.7 ± 2.1%, 54.0 ± 4.6%, and 55.7 ± 3.8% of the LV at 7, 60, and 180 days post-MI, respectively; \( P = \text{NS} \)). In contrast, a progressive and significant reduction of infarct size was observed in hearts harvested from MHC-cycD2 mice (infarct size was 53.4 ± 2.4%, 41.1 ± 3.1%, and 34.9 ± 3.6% of the LV at 7, 60, and 180 days post-MI, respectively; \( P < 0.05 \) for day 60 and day 180 vs. day 7). Importantly, the presence of similar infarct size in non-transgenic and MHC-cycD2 hearts at 7 days post-MI indicated that transgene expression was not cardioprotective acutely.

3.2 Histological analysis and calcium transients of newly formed myocardium

Gross analysis of sections prepared from the non-transgenic hearts revealed that the myocardial content of the infarcted region remained largely unchanged between 7 and 180 days post-MI (Figure 1A). In contrast, a large portion of the scar tissue present at 7 days post-MI was replaced with myocardial tissue by 180 days post-MI in the MHC-CycD2 transgenic mice (Figure 1A). This newly formed myocardium was observed to envelop scar tissue in sections prepared from apical regions of the heart (Figure 1A, arrows). Moreover, scar tissue was largely resolved in sections located near the base of the heart (Figure 1A, arrowheads). Microscopic examination of the newly formed myocardium at the apical scar/myocardial border of MHC-cycD2 hearts at 180 days post-MI revealed the presence of cardiomyocytes with well-organized sarcomeres (Figure 1B, left panel). Connexin43 (a major component of the cardiac gap junction) immune reactivity was readily detected between cardiomyocytes in this region (Figure 1B, right panel).

The presence of connexin43 immune reactivity at junctional complexes between adjacent cardiomyocytes raised the possibility that the newly formed myocardium might participate in a functional syncytium with the surviving remote myocardium. To directly test this, hearts from MHC-cycD2 mice harvested at 180 days post-MI were placed on a Langendorff apparatus, perfused with the calcium sensing dye rhod-2, and imaged using TPME laser scanning microscopy. This assay permitted direct monitoring of intracellular calcium ([Ca\(^{2+}\)]\(_i\)) transients in intact hearts.18 The hearts were imaged at the apical scar/myocardial border (i.e. the anatomical position of the newly formed myocardium). Periodic increases in rhod-2 fluorescence, due to spontaneous action potential-evoked

Figure 1 Characterization of myocardial infarcts in non-transgenic and MHC-cycD2 mice. (A) Representative Azan staining of coronal sections from non-transgenic and MHC-cycD2 hearts at 7 and 180 days post-myocardial infarction. The sections were sampled at 2, 3, and 4 mm from the apex of the heart, as indicated. Arrows indicate regions of newly formed myocardium in the MHC-cycD2 heart at 180 days post-myocardial infarction, which were not present in the corresponding anatomical location at 7 days post-myocardial infarction, nor in the non-transgenic mice. Arrowheads indicate where the infarct scar has been largely resolved near the base of the heart by 180 days post-myocardial infarction in the MHC-cycD2. (B) Consecutive sections of the apical scar/myocardial border of an MHC-cycD2 mouse at 180 days post-myocardial infarction stained with Azan (left panel) and connexin-43 (right panel; HRP-conjugated secondary antibody). Arrow indicates the junctional complex between two cardiomyocytes within the newly formed myocardium; SC, scar; MY, myocardium.
elevations in cytosolic calcium concentration, were visible as ripple-like wave fronts in the cardiomyocytes, but not in the adjacent scar tissue, in images obtained in TPME full-frame mode (Figure 2A).

To better monitor the temporal changes in \([\text{Ca}^{2+}]_i\), fluorescence signals were also recorded in line-scan mode during normal sinus rhythm. The scan line (Figure 2A, white bar) traversed three cardiomyocytes in the newly formed myocardium. This line was repeatedly imaged and the resulting line scans were stacked vertically (Figure 2B). Averaged traces of the intensity of the fluorescence signal from the cardiomyocytes were then generated from the line-scan data (Figure 2C). Cardiomyocytes located in the newly formed myocardium exhibited transient increases in rhod-2 fluorescence (corresponding to spontaneous action potential-evoked increases in \([\text{Ca}^{2+}]_i\)) in synchrony with one another as well as with the remote myocardium. To examine \([\text{Ca}^{2+}]_i\) transient duration, changes in fluorescence for individual cells were normalized such that 0 represented the fluorescence value prior to \([\text{Ca}^{2+}]_i\) transient onset and 1 represented the peak fluorescence value. Superimposition of normalized \([\text{Ca}^{2+}]_i\) transients revealed that \([\text{Ca}^{2+}]_i\) transient duration in individual cardiomyocytes within the newly formed myocardium were similar to one another and moreover were similar to those recorded in remote cardiomyocytes (Figure 2D). Thus, newly formed myocardium in infarcted MHC-cycD2 transgenic hearts appeared to participate in a functional syncytium with the surviving remote myocardium.

### 3.3 Cardiac function

To determine whether the presence of coupled, newly formed myocardium had a positive impact on cardiac function, LV pressure–volume measurements were compared between sham-operated and infarcted non-transgenic mice, and between sham-operated and infarcted MHC-cycD2 mice, at 7, 60, and 180 days post-surgery. For sham surgery, the chest and pericardium were opened but the coronary artery was not occluded. As expected, MI resulted in a marked and statistically significant deterioration in many physiological parameters of the non-transgenic mice at 7 days post-MI compared with the corresponding sham-operated animals (Table 1). No improvement in cardiac function was apparent at 60 and 180 days post-MI, consistent with the absence of improved cardiac architecture at these time points in the non-transgenic animals.

Cardiac function was similarly reduced in the MHC-cycD2 hearts at 7 days post-MI compared with the sham-operated animals (Table 2), in agreement with the deterioration of cardiac architecture seen at this time point. However, marked improvements of functional parameters in the transgenic mice were observed at later time points post-MI (Table 2). Improvement in the LV peak positive pressure rise rate-end diastolic volume slope \((dP/dt_{\text{max}}/EDV)\) was particularly noteworthy, as this parameter provided a highly reproducible and load-independent index of myocardial contractility. At 180 days post-MI, all parameters measured were not statistically different in infarcted...
Table 1  Haemodynamic parameters in non-transgenic mice at 7, 60, and 180 days after sham-operation or myocardial infarction

<table>
<thead>
<tr>
<th></th>
<th>7 day sham (n = 11)</th>
<th>7 day MI (n = 12)</th>
<th>60 day sham (n = 9)</th>
<th>60 day MI (n = 9)</th>
<th>180 day sham (n = 9)</th>
<th>180 day MI (n = 8)</th>
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<tr>
<td>HR (b.p.m.)</td>
<td>525 ± 7</td>
<td>534 ± 30</td>
<td>520 ± 10</td>
<td>517 ± 10</td>
<td>494 ± 10</td>
<td>458 ± 17</td>
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<td>Pes (mmHg)</td>
<td>57.5 ± 1.3</td>
<td>56.7 ± 1.8</td>
<td>62.8 ± 3.3</td>
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<td>58.6 ± 2.8</td>
<td>56.7 ± 2.3</td>
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<tr>
<td>Ped (mmHg)</td>
<td>2.2 ± 0.2</td>
<td>3.8 ± 0.9</td>
<td>3.1 ± 0.4</td>
<td>3.8 ± 0.8</td>
<td>2.0 ± 0.5</td>
<td>3.6 ± 0.5*</td>
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<td>dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td>6105.6 ± 222.9</td>
<td>3736.6 ± 169.7*</td>
<td>6627.1 ± 147.7</td>
<td>3322.3 ± 164.7*</td>
<td>5794.8 ± 391.8</td>
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<td>dP/dt&lt;sub&gt;min&lt;/sub&gt; (mmHg/s)</td>
<td>-3916.3 ± 86.8</td>
<td>-3328.8 ± 345.0</td>
<td>-4117.3 ± 124</td>
<td>-2007.2 ± 206.6*</td>
<td>-3950.6 ± 200.1</td>
<td>-2861.6 ± 135.7*</td>
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<tr>
<td>Tau (ms)</td>
<td>10.1 ± 0.3</td>
<td>12.5 ± 0.7*</td>
<td>10.0 ± 0.3</td>
<td>14.2 ± 0.8*</td>
<td>10.6 ± 0.5</td>
<td>13.8 ± 0.5*</td>
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<tr>
<td>ESPVR (mmHg/μL)</td>
<td>41.1 ± 3.2</td>
<td>23.9 ± 2.0*</td>
<td>36.2 ± 3.7</td>
<td>14.3 ± 1.9*</td>
<td>27.3 ± 5.1</td>
<td>16.0 ± 3.0</td>
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<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;/EDV (mmHg/s/μL)</td>
<td>2095.3 ± 200.2</td>
<td>1094.4 ± 158.8*</td>
<td>1721.7 ± 200.0</td>
<td>636.0 ± 142.8*</td>
<td>1229.2 ± 177.8</td>
<td>520.2 ± 106.7*</td>
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</table>

*P < 0.05, MI mice vs. their respective sham-operated controls.

Table 2  Haemodynamic parameters in MHC-cycD2 transgenic mice at 7, 60, and 180 days after sham-operation or myocardial infarction

<table>
<thead>
<tr>
<th></th>
<th>7 day sham (n = 12)</th>
<th>7 day MI (n = 10)</th>
<th>60 day sham (n = 12)</th>
<th>60 day MI (n = 12)</th>
<th>180 day sham (n = 10)</th>
<th>180 day MI (n = 8)</th>
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<tr>
<td>HR (b.p.m.)</td>
<td>524 ± 9</td>
<td>502 ± 11</td>
<td>516 ± 7</td>
<td>526 ± 7</td>
<td>486 ± 11</td>
<td>496 ± 20</td>
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<tr>
<td>Pes (mmHg)</td>
<td>62.5 ± 4.7</td>
<td>58.2 ± 3.5</td>
<td>62.2 ± 2.1</td>
<td>61.8 ± 2.1</td>
<td>593.3 ± 2</td>
<td>662.2 ± 5.2</td>
</tr>
<tr>
<td>Ped (mmHg)</td>
<td>2.7 ± 0.4</td>
<td>3.5 ± 0.7</td>
<td>3.0 ± 0.5</td>
<td>3.6 ± 0.6</td>
<td>2.8 ± 0.6</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td>6132.5 ± 174.7</td>
<td>3459.9 ± 295.8*</td>
<td>6563.2 ± 152.0</td>
<td>4181.5 ± 339.8*</td>
<td>5865.0 ± 215.5*</td>
<td>4961.8 ± 731.8</td>
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<td>dP/dt&lt;sub&gt;min&lt;/sub&gt; (mmHg/s)</td>
<td>-4079.8 ± 228.3</td>
<td>-3077.8 ± 318.0*</td>
<td>-4117.0 ± 79.5</td>
<td>-3521.3 ± 270.8*</td>
<td>-3894.8 ± 131.4</td>
<td>-3719.2 ± 397.2</td>
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<tr>
<td>Tau (ms)</td>
<td>10.1 ± 0.3</td>
<td>11.9 ± 0.3*</td>
<td>10.2 ± 0.2</td>
<td>12.2 ± 0.49*</td>
<td>10.9 ± 0.4</td>
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<td>ESPVR (mmHg/μL)</td>
<td>45.5 ± 4.4</td>
<td>26.3 ± 5.6*</td>
<td>38.3 ± 3.7</td>
<td>29.6 ± 2.3</td>
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<td>29.0 ± 3.1</td>
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<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;/EDV (mmHg/s/μL)</td>
<td>1878.5 ± 114.1</td>
<td>873.7 ± 150.4*</td>
<td>1952.7 ± 104.3</td>
<td>1320.7 ± 116.7*</td>
<td>1336.1 ± 119</td>
<td>1172.7 ± 222.7</td>
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</table>

HR, heart rate; Pes, left ventricular end-systolic pressure; Ped, left ventricular end-diastolic pressure; dP/dt<sub>max</sub>, left ventricular positive pressure rise rate; dP/dt<sub>min</sub>, left ventricular peak negative pressure rise rate; Tau, left ventricular isovolumic relaxation time constant; ESPVR, end-systolic pressure-volume relation; dP/dt<sub>max</sub>/EDV, slope of the left ventricular positive pressure rise rate with respect to volume. *P < 0.05, MI mice vs. their respective sham-operated controls.
MHC-cycD2 mice compared with MHC-cycD2 mice with sham surgery, indicating a remarkable degree of functional recovery in the transgenic hearts. The impact of transgene expression on cardiac function was even more apparent when the parameters measured in infarcted mice were normalized to those in the corresponding sham-operated animals (Figure 3); all parameters tested in the MHC-cycD2 mice improved with time. In contrast, physiological parameters either did not improve or deteriorated in the non-transgenic animals.

4. Discussion

Previous studies indicated that cardiomyocyte-restricted cyclin D2 expression resulted in regenerative growth in injured hearts, as evidenced by increased cardiomyocyte number and concomitant reduction of scar tissue mass at 150 days post-MI. The data presented here demonstrated that regenerative cardiac growth was present as early as 60 days post-MI in MHC-cycD2 transgenic mice. Moreover, cardiomyocytes in the newly formed myocardium expressed connexin43 and were functionally coupled with one another and with the remote myocardium as demonstrated by TPME imaging of [Ca^2+], transients. Cardiac functional parameters improved in the infarcted MHC-cycD2 hearts, but not in the non-transgenic siblings, compared with their respective sham-operated controls. Importantly, the degree of functional improvement in infarcted MHC-cycD2 mice correlated directly with reduction in infarct size (and concomitantly, the newly formed myocardium content) at 60 vs. 180 days post-MI.

To date, only a limited number of proteins have been shown to induce sustained ventricular cardiomyocyte cell cycle activity when expressed in adult transgenic animals. These include SV40 Large T antigen, an inducible form of c-myc, CDK-2, dominant interfering TSC2, dominant interfering p193, cyclin A2, IGF-1, and bcl-2. Of these, only dominant interfering p193 (an E3 ubiquitin ligase molecule originally identified as an SV40 large T antigen-binding protein) and IGF-1 have so far been shown to improve cardiac function following myocardial injury. In the case of the dominant interfering p193 mice, cardiomyocyte cell cycle activity was induced following MI, resulting in more favourable post-MI ventricular remodelling and a concomitant improvement in cardiac function. In the case of the IGF-1 mice, it was not clear whether the beneficial effect on cardiac function resulted from transgene-induced cardiomyocyte proliferation or alternatively from reduced levels of cardiomyocyte apoptosis. Paradoxically, although cardiomyocyte cell cycle activity was induced in adult mice over-expressing CDK-2, these animals exhibited an aberrant hypertrophic response to surgically induced pressure overload. Importantly, none of these studies demonstrated progressive restoration of cardiac structure and function post-injury, which would be consistent with regenerative growth of the myocardium.

In contrast, the data presented here indicate that MHC-cycD2 mice undergo a progressive improvement in both cardiac structure and function following MI. Given that the MHC-promoter is restricted to differentiated cardiomyocytes, and given the high rates of cardiomyocyte DNA synthesis observed previously following MI, the simplest interpretation for the improvement of cardiac architecture and function observed in the current study is transgene-induced proliferation of pre-existing cardiomyocytes. It is however possible that transgene expression may also enhance the reparative capacity of putative cardiomyogenic progenitor cells. For example, adult stem cell-
derived cardiomyocytes would also likely exhibit enhanced cell cycle activity, provided that they express alpha MHC promoter (and concomitantly, the MHC-cycD2 transgene). The use of a conditional transgene system is required to quantify the relative contributions of pre-existing cardiomyocytes and cardiomyogenic stem cells observed in the current study. Regardless of the mechanistic origin of the de novo myocardium observed here, it is noteworthy that the magnitude of cardiac functional improvement following MI in the MHC-cycD2 hearts compared quite favourably with that obtained in most experimental studies using adult cardiomyogenic stem cell-based transplantation or mobilization interventions. Indeed, the reported impact of stem cell intervention on cardiac structure and cardiac function has been highly variable, ranging from considerable to no detectable impact, despite the use of similar cell types, injury models, and experimental read-outs. Importantly, functional improvement in the injured MHC-cycD2 transgenic hearts persisted for 180 days post-MI. It is of interest to note that the degree of infarct reduction in MHC-cycD2 at 180 days post-MI (35%) was somewhat greater than that observed in our previous study, which was analysed at 150 days post-MI. This is likely to be attributable at least in part to the longer duration of the experiment.

Modulation of cyclin D activity in cardiomyocytes after myocardial injury may have clinical implications for cardiac regeneration. For example, cyclin D2 gene transfer in human myocardium could possibly lead to a gene-based regenerative mechanism in patients. In support of this, in vivo experiments revealed that genetically naïve adult rat cardiomyocytes respond to cyclin D following adenosivial gene transfer. Although our model utilized a rather strong, constitutively active promoter, the level of transgene-derived cyclin D2 expression in the adult mice was similar to that seen for the endogenous cyclin D2 gene in foetal hearts when similar levels of total protein were compared. Unfortunately, similar levels of transgene expression were observed in the different MHC-cycD2 lineages we generated, precluding the establishment of a dose–response relationship between the level of cyclin D expression and regenerative growth. Although cell cycle activity persisted in aged MHC-cycD2 mice, disorganized tumour-like growth has not been observed, and the hearts remained comprised of well-differentiated cardiomyocytes. Nonetheless, the use of constitutively active promoters to target growth-promoting genes would not be appropriate for therapeutic applications. Perhaps more realistically, development of pharmacological agents capable of modulating cyclin D expression and/or activity in cardiomyocytes might prove to be a useful approach to engender regenerative cardiac growth. Regardless of the mechanism employed, cardiomyocyte cell cycle induction may represent an important therapeutic tool for cardiac regeneration and enhancement of cardiac function after MI.

Acknowledgements

We thank Dr Paul Steendijk (Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands) for assistance with pressure-volume data analysis.

Conflict of interest: none declared.

Funding

This work has been supported by the Hein J.J. Wellens Foundation and the Foundation De Drie Lichten (both in The Netherlands) and by grants from the Heart Lung and Blood Institute of the National Institutes of Health (USA).

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