

Underlying mechanisms and prospects of heart regeneration

Galip Servet ASLAN, Dudu Gonca MISIR, Fatih KOCABAŞ*

Department of Genetics and Bioengineering, Regenerative Biology Research Laboratory, Yeditepe University, İstanbul, Turkey

Received: 03.06.2015 • Accepted/Published Online: 13.09.2015 • Final Version: 23.02.2016

Abstract: Findings in the last decade suggest that there is a considerable amount of cardiomyocyte turnover in the human heart throughout life, albeit not sufficient for heart regeneration following myocardial infarctions. Only a few species are known to be remarkably efficient in cardiac regeneration. They restore lost cardiomyocytes via a process of cardiomyocyte dedifferentiation, which is followed by robust proliferation of cardiomyocytes and incorporation into the myocardium. Similarly, neonatal mice have been recently shown to regenerate their heart following myocardial injuries. Studies with a neonatal cardiac regeneration mouse model suggest that the major source of new cardiomyocytes is likely to be of cardiomyocyte origin, with the possibility of involvement of cardiac stem cells. To this end, numerous studies have been conducted on the induction of cardiac regeneration to shed light on the underlying mechanisms. This review covers studies on the renewal of cardiomyocytes, the utilization of stem cells in myocardial therapies, and their future applications.

Key words: Cardiomyocyte renewal, cardiogenic factors, cardiopoietic factors, cardiomyocyte proliferation, resident heart stem cells

1. Introduction

Heart failure (HF) is a complex clinical syndrome associated with decreased function of the ventricle to fill or eject blood, and affects more than 23 million people worldwide (Jessup et al., 2009). Unfortunately, heart transplantation in the presence of an appropriate donor remains the only definitive treatment for HF (Jessup and Brozena, 2003).

Cardiac regeneration is a multidisciplinary research area comprising physiology, stem cell, developmental biology, and tissue engineering, and has the ultimate goal of reversing heart failure in the context of regenerative medicine. Over the past decade, there has been mounting evidence that the heart is certainly not a terminally differentiated organ. There is a constant cardiomyocyte turnover within the mammalian (and the human) heart throughout life (Laflamme et al., 2002; Bergmann et al., 2009; Kajstura et al., 2010; Bergmann et al., 2012). Evidence for the role of cardiac resident stem cells, cardiomyocyte proliferation, and exogenous stem cells has been presented in back to back reports (Orlic et al., 2001; Beltrami et al., 2003; Jopling et al., 2010; Kikuchi et al., 2010). However, revealing the cardiac regenerative capacity of the human heart and designing robust therapeutical strategies require learning more about the molecular mechanism of cardiac regeneration. To this end, studies on model organisms

such as zebrafish, newt, and murine heart provide great opportunity to elucidate the underlying mechanism and recipe for cardiac regeneration.

Here we review the literature on the concepts important in the field of cardiac regeneration. First, we discuss cardiac regeneration studies in model organisms, including zebrafish and newt. Second, we explain the recently reported neonatal mice cardiac regeneration model and its mechanism to understand the hurdles of mammalian cardiac regeneration. Third, we discuss studies in human subjects that indicate the existence of cardiomyocyte turnover in the human heart. Finally, we discuss the contribution of cardiac resident stem cells and the proliferation of cardiomyocytes and bone marrow derived stem cells into cardiac regeneration.

2. Heart regeneration

Heart regeneration in lower vertebrates has been intensively studied. The regenerative potential of vertebrate hearts including amphibian, axolotls, and newts was identified in early reports (Rumyantsev, 1966; Sulima, 1968; Rumyantsev, 1973). Using electron microscopy in 1974, Oberpriller et al. (1974) demonstrated the prospect of cardiac regeneration in newts. Later, Witman et al. (2011) reported that the adult newt is able to completely regenerate its heart after a basal resection (Witman et al., 2011). Zebrafish (*Danio rerio*) is a tropical freshwater fish

* Correspondence: fatih.kocabas@yeditepe.edu.tr

that has been widely used in many different regenerative studies, including those of the heart (Poss et al., 2002; Jopling et al., 2010; Kikuchi et al., 2010), kidney (Sander and Davidson, 2014), and central nervous system (Becker and Becker, 2008). The complete regeneration of zebrafish heart after amputation of the ventricular apex has already been reported (Poss et al., 2002) and has led to new studies to understand the mechanisms of cardiac regeneration (Wills et al., 2008; Jopling et al., 2010; Kikuchi et al., 2010; Gemberling et al., 2015). On the other hand, the utilization of a recently developed cardiac injury model in 1-day-old neonatal mice revealed that the neonatal mouse heart is also capable of regeneration after apical resection of 15% of the ventricular apex (Porrello et al., 2011; Mahmoud et al., 2014). Many different studies regarding the mechanism of cardiac regeneration in mammals were reported to elucidate the underlying mechanisms of heart regeneration. Here, we review studies regarding cardiac regeneration in zebrafish, newts, and neonatal mice (Table 1).

2.1. Heart regeneration in zebrafish

Heart regeneration has been observed in nonmammalian vertebrate hearts such as in salamanders and zebrafish

(Oberpriller and Oberpriller, 1974; Neff et al., 1996; Flink, 2002; Poss et al., 2002; Raya et al., 2003). Zebrafish have become one of the major model organisms for the study of cardiac regeneration over the past decade. This is largely due to the visibility of structures during development, easy access to the heart to perform surgical operations, a large number of offspring, and low cost of maintenance. In addition, an intact cardiovascular system in a zebrafish embryo is not required, which allows the investigation of cardiac regeneration without causing the death of zebrafish embryos (Pelster and Burggren, 1996). Poss et al. (2002) demonstrated zebrafish cardiac regeneration following an amputation of up to 20% of the ventricle by surgical resection (Poss et al., 2002). This operation initially led to fibrosis, followed by the complete regeneration of lost tissue in 60 days. In addition to the resection of the heart, other means of injury in zebrafish have been shown to provide tools to study different aspects of heart regeneration with different degrees of regenerative response (González-Rosa et al., 2011; Wang et al., 2011).

The cryoinjury method, for instance, depends on the induction of injury by a liquid nitrogen probe on the

Table 1. Various cardiac injury methods in different organisms and their regenerative response.

Organism	Zebrafish 	Newt 	Neonatal Mice 	Adult Mice 	Human 
Injury or treatment	Apical resection	Apical resection	Apical resection	Apical resection (7 day old mouse)	-
	Cryoinjury	Basal resection	Cryoinjury	Cryoinjury	Use of LVAD
	Genetic ablation	-	Ischemic MI	Ischemic MI	Ischemic MI
Response and time	Regeneration in 60 days	(Incomplete) regeneration	Regeneration in 21 days	Fibrosis & no regeneration	-
	Regeneration > 130 days	Regeneration in 60 days	Regeneration in 21 days	Fibrosis & no regeneration	Fibrosis & no regeneration (modest CM proliferation)
	Regeneration in 30–45 days	-	Regeneration in 21 days	Fibrosis & no regeneration	Fibrosis & no regeneration
References	(Poss et al., 2002) (González-Rosa et al., 2011) (Jopling et al., 2010) (Kikuchi et al., 2010) (Chablais et al., 2011) (Wang et al., 2011) (Schnabel et al., 2011)	(Oberpriller and Oberpriller 1974) (Witman et al., 2011)	(Porrello et al., 2011) (Porrello et al., 2013) (Mahmoud et al., 2014) (Mahmoud et al., 2013) (Darehzereshki et al., 2014)	(Porrello et al., 2011) (Porrello et al., 2013) (Mahmoud et al., 2014) (Mahmoud et al., 2013)	(Kajstura et al., 2010) (Canseco et al., 2015)

heart. When this procedure was performed in zebrafish, it led to heart regeneration over a longer time period (more than 130 days) (Chablais et al., 2011; González-Rosa et al., 2011). Wang et al. (2011) applied a genetic ablation injury to extrapolate the regenerative response of zebrafish hearts. They have selectively expressed the diphtheria toxin gene A (DTA) in cardiomyocytes by Cre-loxP mediated recombination, which is under the control of a cardiac myosin light chain 2 promoter (*cmcl2*). This led to the genetic ablation and death of cardiomyocytes, albeit resulting in complete regeneration of the heart in 45 days (Wang et al., 2011). These findings prompted studies on the identification of the origin of newly formed cardiomyocytes.

The source of newly grown cardiomyocytes following myocardial injury in zebrafish has been a subject of debate. Many researchers used to think that new cardiomyocytes following cardiac injury in zebrafish originated from progenitor cells instead of preexisting cardiomyocytes (Lepilina et al., 2006). However, two landmark studies provided evidence that newly formed cardiomyocytes in the cardiac regeneration process were largely derived from preexisting cardiomyocytes (Jopling et al., 2010; Kikuchi et al., 2010). Integration of the Cre recombinase system as well as the GFP inducible genetic lineage tracing method in zebrafish led to tracking the origin of newly formed cardiomyocytes in the apical injury model following the removal of 20% of the ventricular apex (Jopling et al., 2010; Kikuchi et al., 2010). Expression of GFP in newly formed cardiomyocytes provided evidence that they arise from preexisting ones. Furthermore, this study suggested a dedifferentiation mechanism, which was measured by the disassembly of the sarcomeric organization of cardiomyocytes, and initiated cardiomyocyte cell cycle progression and proliferation. Moreover, this cell cycle progression has been regulated by polo like kinase 1 (*plk1*), an important cell cycle modulator (Jopling et al., 2010).

Kikuchi et al. (2010) provided further evidence on the origin of newly formed cardiomyocytes, which relied on GATA4 expression. GATA4 was expressed in cardiomyocytes of the subepicardial ventricular layer and proliferating cells near the site of injury (Molkentin et al., 1997; Pu et al., 2004; Zeisberg et al., 2005). Kikuchi et al. (2010) utilized *gata4*-EGFP and demonstrated that myocyte marker carrying cells expressed GFP. On the other hand, epicardium marker carrying cells did not express GFP 2 weeks postinjury. These results suggest that cardiomyocytes existing near the injury site are triggered to express GATA4 and reactivate the cardiomyocyte cell cycle and proliferation (Kikuchi et al., 2010).

2.2. Heart regeneration in newt

Notophthalmus viridescens, a kind of red-spotted newt, is classified in the urodele amphibians and is commonly

accepted as the champion of regeneration. Regenerative biology studies in newts have established the extraordinary regenerative ability of various organs and tissues including limbs, tail, lenses, spinal cord, and heart (Witman et al., 2011). The newt heart is located close to the skin and consists of three chambers (two atria and one ventricle). Trabeculae form the newt cardiac ventricle, where a single layer of epicardial cells surrounds myocytes, fibroblasts, and nerve fibers (Singh et al., 2010). Initial reports on newt cardiac regeneration date back to the 1970s. In 1974 Oberpriller et al. showed the mitotic response of the newt heart and the possibility of newt cardiac regeneration. Although underlying studies did not report the complete regeneration of the newt heart after amputation of one-eighth of the ventricular apex, they led to increased mitosis in the heart (Bader and Oberpriller, 1979; Borchardt and Braun, 2007). Thus, studies were rather performed with a modified apical resection model, in which the ventricular cavity was left intact and investigated for a longer period to assess regenerative response. In this case, complete cardiac ventricular regeneration was observed in 60 days along with an increased expression of cardiac specific transcription factors such as GATA4, Nkx-2.5, GATA5, Islet1, and HAND2 at the peak of proliferation. This indicates that a proliferative response is achieved by a coordinated expression of transcription factors (Witman et al., 2011). In addition, following cardiac injuries in newts, there is a change in the expression of extracellular matrix (ECM) related genes instead of metabolic or inflammation related ones. The expression of ECM genes, such as collagen III and tenascin-C, increases just after amputation or injury of the ventricular apex. This suggests that the reorganization of the ECM is involved in the replenishment of lost cardiomyocytes (Mercer et al., 2013; Piatkowski et al., 2013). Moreover, Mercer et al. (2013) reported that tenascin-C significantly increases the reentry of cardiomyocytes into the cell cycle in vitro (Mercer et al., 2013).

2.3. Heart regeneration in mammals

It was thought that the total number of cardiomyocytes in a mammalian heart is set at birth and does not change through life. However, over the past decade it was demonstrated that the heart is certainly not a terminally differentiated organ (Laflamme et al., 2002; Bergmann et al., 2009; Kajstura et al., 2010; Bergmann et al., 2012). A number of recent studies provided strong evidence for cardiomyocyte renewal in the human heart (Laflamme et al., 2002; Quaini et al., 2002; Kajstura et al., 2010; Bergmann et al., 2012b). Bergmann et al. (2009) utilized an elegant approach to determine the age of cardiomyocytes, thus determining if any cardiomyocyte turnover occurs in the human heart. The pulse-chase conditions of ¹⁴C levels in the atmosphere due to testing of nuclear weapons

during the Cold War enabled the measurement of the age of cardiomyocytes in human subjects (who were older than 20 years). Analysis of the ^{14}C content and turnover in cardiomyocytes indicated that cardiomyocytes were younger than expected, which suggested that they were not set at birth. Further analysis and mathematical modeling indicated that about 1% of cardiomyocytes are renewed per year at the age of 20 and 0.4% at the age of 75. This provided an estimation of 40%–50% cardiomyocyte renewal in an average human lifespan (Bergmann et al., 2009).

Kajstura et al. (2010) provided further evidence of cardiomyocyte renewal by utilizing samples from postmortem hearts of thymidine analogue iododeoxyuridine (IdU) treated cancer patients. Analysis of IdU incorporation and turnover in the heart indicated the presence of 22% cardiomyocyte turnover per year, which is higher than the estimations reported by Bergmann et al. (Kajstura et al., 2010). The discrepancy between these two studies has been thought to be due to the age and distribution of human subjects. Mollova et al. (2013) recently outlined that there are distinct, age-dependent cardiomyocyte division rates (0.016% in 0–1 year olds, 0.01% in 2–10 year olds, and 0.005% in 10–20 year olds) (Mollova et al., 2013).

In another study, male patients who received hearts from female donors were subjected to chimerism analysis of the transplanted heart. Depending on the Y chromosome analysis and immunolabeling of recipient primitive cells, a contribution of primitive cell migration from the recipient to the graft area was noted; thus chimerism of the heart was reported (Quaini et al., 2002). Moreover, Shiba et al. (2012) reported that integrated cardiomyocytes, derived from human embryonic stem cells, could be used against arrhythmias in a guinea pig model. Besides previous findings suggesting that transplantation of fetal cardiomyocytes can improve the function of infarcted hearts, this was a landmark study regarding cell replacement therapies for cardiovascular disease (Li et al., 1996; Caspi et al., 2007; Laflamme et al., 2007; Shiba et al., 2012)

Studies have shown that the DNA damage response due to a high oxygen environment during postnatal mice exposure is an important mechanism in cardiomyocyte cell cycle arrest (Puente et al., 2014). A recent study investigated the effect of the left ventricular assist device (LVAD) on mitochondrial content and cardiomyocyte proliferation (Canseco et al., 2015). By comparing pre-LVAD and post-LVAD patients, it was demonstrated that prolonged mechanical unloading causes up to 60% decrease in mitochondrial mass and ROS, as well as about a threefold increase in cardiomyocyte proliferation (Canseco et al., 2015).

Examination of the regenerative capacity of the mouse heart took cardiac regeneration studies a step further. Mouse cardiomyocytes are highly proliferative during embryogenesis. At postnatal day 4 cardiomyocytes dramatically lose this proliferative capacity. Cardiomyocytes undergo karyokinesis without the cytokinesis step, thus resulting in binucleation of 90% of adult cardiomyocytes (Li et al., 1996; Soonpaa et al., 1996; Walsh et al., 2010). Mammals differ from other vertebrates in terms of cardiac regeneration capacity by possessing mostly binucleated cardiomyocytes, a greater heart volume, four chambered hearts, high pressure containing blood flow, and an associated complex genome (Fishman and Olson, 1997). Similar to zebrafish, one of the debated issues was the source of newly formed cardiomyocytes in mammals that were recently revealed by rodent and human studies (Porrello et al., 2011; Senyo et al., 2013).

Mice possess a low rate of cardiomyocyte turnover as confirmed by various studies and different approaches. BrdU incorporation and quantification by an anti-BrdU antibody were utilized to assess cell proliferation in the mouse heart. Similarly, a thymidine analogue (thymidine [^3H]) was incorporated into newly formed DNA strands as the cell cycle progressed and was used as a marker for cardiomyocyte proliferation. Another approach utilized the incorporation of ^{15}N , coupled with multiisotope imaging mass spectrometry (MIMS), to assess proliferating cells in the heart. These studies indicated that an adult mouse shows 0.74%–4.5% of an unstimulated rate of cardiomyocyte renewal (Table 2) (Soonpaa et al., 1996; Soonpaa and Field, 1997; Malliaras et al., 2013; Senyo et al., 2013).

The source of newly formed cardiomyocytes in mammals has been the subject of debate for years (Laflamme and Murry, 2011). Recent studies demonstrated that proliferation of preexisting cardiomyocytes occurs in mice after myocardial infarction and reported that cardiac progenitor cells have a modest effect, as suggested in previous studies (Hosoda et al., 2009; Malliaras et al., 2013; Senyo et al., 2013). However, the possibility of a progenitor or stem cell population to be involved in cardiac regeneration following injury through differentiation has not been excluded. Along with the resident capacity to replenish cardiomyocytes, recent studies, especially in neonatal animals, suggest that mammalian hearts possess a hidden regeneration potential.

2.4. Heart regeneration in neonatal mouse

Compared with zebrafish and newt, the adult mammalian heart has a limited capacity for cardiomyocyte renewal following injury, and responds to cardiac tissue damage by scar formation. As we have explained earlier, mouse cardiomyocytes undergo dramatic changes during the first week of life, marked by the expression of adult isoforms of

Table 2. Unstimulated rate of cardiomyocyte renewal in different species.

Species	Estimated cardiomyocyte renewal/year	Methods	Sources of newly formed cardiomyocytes	References
Zebrafish	ND	ND	Largely cardiomyocytes but it does not exclude the involvement of cardiac progenitors	(Jopling et al., 2010; Kikuchi et al., 2010)
Mouse	0.74%–4.5%	BrdU, ¹⁵ N, imaging mass spectrometry, [³ H] thymidine	Largely cardiomyocytes but it does not exclude the involvement of cardiac progenitors	(Soonpaa et al., 1997; Malliaras et al., 2013; Senyo et al., 2013)
Human	0.04%–40%	¹⁴ C, accelerator mass spectrometry, Ki67, phospho-H3, Aurora B, and IdU	Largely cardiomyocytes but it does not exclude the involvement of cardiac progenitors	(Kajstura et al., 2010; Bergmann et al., 2012; Mollova et al., 2013)

ND: not determined.

contractile proteins and the induction of DNA synthesis without cytokinesis, resulting in binucleation and cell cycle arrest at G₀/G₁ (Soonpaa et al., 1996). A recent landmark study accomplished a similar injury model of zebrafish in mice and demonstrated that a 1-day-old neonatal mouse is capable of heart regeneration (Porrello et al., 2011). One study reported the complete regeneration of a neonatal mouse heart without a visible scar and fibrosis following amputation of 15% of the ventricle (Porrello et al., 2011). Another study permanently ligated the left anterior descending (LAD) coronary artery of 1-day-old mice, thus inducing an ischemic myocardial infarction (Porrello et al., 2013). This study further provided evidence that a neonatal mouse following LAD ligation could regenerate the heart in as short as 21 days without obvious fibrosis and scar formation (Porrello et al., 2013). In addition, a Cre-lox inducible genetic fate mapping approach was utilized to address the source of regenerated cardiomyocytes, and showed that newly formed cardiomyocytes arise from preexisting cardiomyocytes after apical resection of the neonatal myocardium (Porrello et al., 2013). Moreover, a cryoinjury model was used to study the regenerative response of neonatal mice hearts. One study indicated that cardiac function did not recover following a transmural (severe) cryoinjury (Darehzereshki et al., 2014). In contrast, although cardiomyocyte proliferation was not robust, nontransmural (mild) cryoinjury allowed for complete recovery of cardiac function (Darehzereshki et al., 2014). These cardiac regeneration models (Figure 1) allowed the identification of cardiomyocyte cell cycle modulators as well as prospective targets to induce heart regeneration.

3. Modulators of cardiomyocyte renewal

Myocardial infarction leads to a substantial loss of cardiomyocytes, which negatively influences cardiac

function. This calls for therapeutic approaches that either preserve existing cardiomyocytes or increase the number of functional cardiomyocytes following myocardial injuries. In this frontier, studies to uncover the mechanism of functional cardiomyocyte renewal became a major point of focus in the context of cardiac therapies. The development of an injury model in the zebrafish (Poss et al., 2002) and neonatal mouse (Porrello et al., 2011) provided a great opportunity to investigate these mechanisms and factors involved in heart regeneration. Over the last decade, contributions of different mechanisms, including transdifferentiation (Orlic et al., 2003; Yeh et al., 2003), dedifferentiation (Jopling et al., 2010; Kikuchi et al., 2010), proliferation of preexisting cardiomyocytes (Jopling et al., 2010; Kikuchi et al., 2010; Senyo et al., 2012), and the role of both cardiac resident stem cells (Beltrami et al., 2003) and bone marrow derived stem cells (Orlic et al., 2001; Kajstura et al., 2005) were suggested as plausible in the treatment of cardiovascular disorders (Figure 2). These studies revealed some of the important factors that modulate cardiac progenitors or the cardiomyocyte cell cycle.

3.1. Cardiogenic factors of cardiomyocytes

Various molecular intervention approaches have been utilized to manipulate cardiomyocyte proliferation (Table 3) (Jackson et al., 1990; Soonpaa et al., 1997; Bicknell et al., 2004; Pasumarthi et al., 2005; Cheng et al., 2007). Jackson et al. (1990) overexpressed c-Myc during embryogenesis of mice and demonstrated both increased cardiomyocyte numbers (almost twofold) and heart weight (Jackson et al., 1990). In another study, adenoviral overexpression of oncogene E1A in cardiomyocytes resulted in induced cardiomyocyte cycling followed by apoptosis (Liu and Kitsis, 1996). Overexpression of cell cycle regulatory proteins has also been tested to see if they enhance cardiomyocyte proliferation. Pasumarthi et al. (2005)

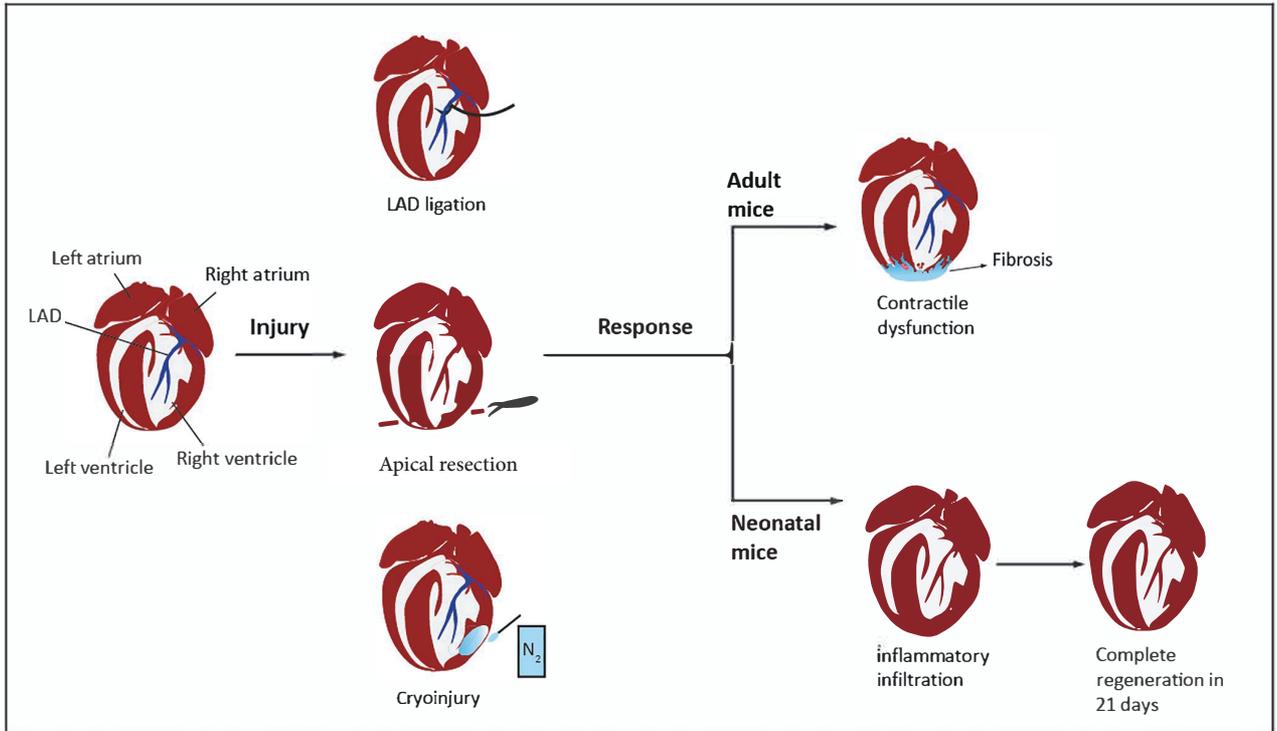


Figure 1. Response to various types of injuries in the mammalian heart. Different injury models were utilized to measure regenerative response of the mammalian heart. The left anterior descending artery (blue) has been permanently ligated in the LAD ligation model. The apical resection injury model leads to an amputation of up to 15% of the ventricular apex. On the other hand, the cryoinjury model induces cardiac damage to the ventricular apex by a pre-cooled probe (cooled by liquid nitrogen). Regenerative responses are quite different in adult and neonatal mice, resulting in contractile dysfunction and complete regeneration, respectively.

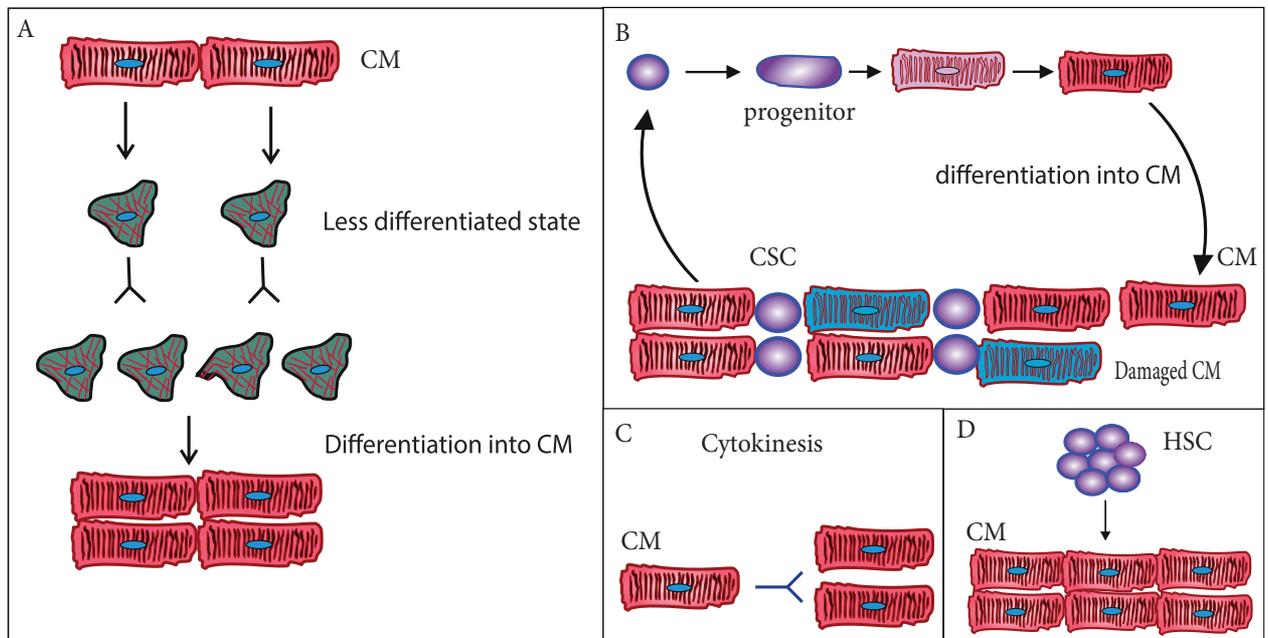


Figure 2. Mechanisms of cardiomyocyte renewal. A) Dedifferentiation initiated by detachment of cardiomyocytes followed by differentiation back into cardiomyocytes; B) cardiac resident stem cells give rise to new cardiomyocytes through differentiation; C) proliferation of preexisting cardiomyocytes; D) transdifferentiation of bone derived hematopoietic stem cells into cardiomyocytes.

Table 3. Major factors involved in cardiomyocyte proliferation.

Cardiogenic factors and manipulations	Fold change in proliferating cardiomyocytes	Reference
Meis1 knockout	9 (pH3 + CMs, Aurora B + CMs)	(Mahmoud et al., 2013)
GSK-3 inhibition (BIO)	5 (pH3 + CMs)	(Tseng et al., 2006)
Foxo1 dominant negative	2.5 (pH3 + CMs)	(Evans-Anderson et al., 2008)
miR-133a knockout	2.5 (pH3 + CMs)	(Liu et al., 2008)
Jumonji knockout	2.2 (pH3 + CMs)	(Jung et al., 2005)
p27 ^{Kip1} knockout	2-3 (troponin I + CMs)	(Poolman et al., 1999)
Constitutively active ERBB2	>12 (Ki67 + CMs, pH3 + CMs, Aurora B+ CMs)	(D'Uva et al., 2015)
Nrg1 (or FGF1, periostin) treatment	>4 (BrdU + CMs, Aurora B + CMs, pH3 + CMs,)	(Bersell et al., 2009) (Gemberling et al., 2015)
Activated Yap1	>7 (Ki67 + CMs, pH3 + CMs, Aurora B+ CMs)	(von Gise et al., 2012)
Salv knockout	>4 (pH3 + CMs)	(Heallen et al., 2011)
IL13 (or IL3, CTGF, Nrg1) treatment	>1.5 (3H Thymidine CMs, Ki67 + CMs, BrdU + CMs)	(O'Meara et al., 2015)
Oncostatin M treatment	>2 (EdU + CMs)	(Kubin et al., 2011)
TWEAK treatment	6.2 (BrdU + CMs)	(Novoyatleva et al., 2010)
C3orf58 treatment	>2 (Ki67 + CMs, BrdU + CMs, Aurora B + CMs)	(Beigi et al., 2013)
Periostin treatment	>5 (BrdU + CMs, pH3 + CMs, Aurora B+ CMs)	(Kühn et al., 2007)
FGF10 treatment	2 (Ki67 + CMs, pH3 + CMs,)	(Rochais et al., 2014)
Cyclin D2 overexpression	>5 (MHC-nLAC + CMs)	(Pasumarthi et al., 2005)
Cyclin B1-CDC2 or cyclin a2 overexpression	>1.4 (CMs)	(Bicknell et al., 2004) (Cheng et al., 2007)
Activated notch	>7 (Ki67 + CMs, BrdU + CMs, Aurora B + CMs)	(Campa et al., 2008)
c-myc or E1A overexpression	2 (CMs)	(Jackson et al., 1990) (Liu and Kitsis, 1996)

CM: cardiomyocytes.

overexpressed cyclin D1, cyclin D2, and cyclin D3, which are considered positive regulators of the G2/M transition state. They reported that neither cyclin D1 nor cyclin D3 increased proliferation of cardiomyocytes after myocardial infarction; however, cyclin D2 overexpression resulted in promoted cardiomyocyte proliferation in adult transgenic mice (Pasumarthi et al., 2005). Successive studies, including forced expression of cyclin B1-CDC2 and transgenic expression of cyclin A2, have resulted in an increased percentage of cardiomyocytes in G2/M *in vitro*, increased cardiomyocyte proliferation, and induced myocardial regeneration in adult mice (Bicknell et al., 2004; Chaudhry et al., 2004). Other studies investigated the downregulation of cell cycle inhibitors such as cyclin-dependent kinase inhibitors (CDKIs) (p21^{Waf1}, p27^{Kip1}, and p57^{Kip2}) due to their high expression in neonatal and adult

heart, and reported an increased cardiomyocyte number (Di Stefano et al., 2011).

Over the past decade, a number of transcription and growth factors involved in the modulation of the cardiomyocyte cycle were identified (Agah et al., 1997; Kühn et al., 2007; Bersell et al., 2009; Kubin et al., 2011; Rochais et al., 2014). The development of the neonatal mouse cardiac regeneration model enabled the investigation of inhibitors of mammalian cardiac regeneration that are activated after the neonatal period (Porrello et al., 2011, 2013; Mahmoud et al., 2013, 2014). Thus, we successfully used this model to identify Meis1, one of the key regulators of neonatal cardiac regeneration, and report that Meis1 inhibits cardiomyocyte proliferation through transcriptional activation of CDKIs p15, p16, and p21 (Mahmoud et al., 2013).

Signaling pathways have been intensively studied to understand and overcome the limited regenerative capacity of the heart (Xin et al., 2013). In this frontier, neuregulin, a ligand for the neuregulin/ErbB2/ErbB4 signaling cascade, was revealed as a positive regulator of cardiomyocyte proliferation (D'Uva et al., 2015) both in overexpression (Gemberling et al., 2015) and recombinant protein administration studies (Gassmann et al., 1995; Lee et al., 1995; Lai et al., 2010; Liu et al., 2010). In addition, the administration of recombinant oncostatin M, TWEAK, FGF10, and periostin, and the coadministration of FGF1 with p38 inhibitor resulted in cardiomyocyte proliferation after myocardial infarction (Engel et al., 2006; Kühn et al., 2007; Novoyatleva et al., 2010; Kubin et al., 2011; Rochais et al., 2014). Several studies on the Hippo signaling pathway demonstrated its prospect in cardiac regeneration. Overexpression of one of the key component of the Hippo pathway, the yes-associated protein (YAP), and the knockdown of an upstream effector element of the Hippo pathway resulted in an increased cardiomyocyte number and a thickening of the myocardial wall (Halder and Johnson, 2011; Heallen et al., 2011; Xin et al., 2011; von Gise et al., 2012; Xiao et al., 2015).

Nerves have been known to guide organ regeneration. However, their function in cardiac regeneration was not determined until recently (Kumar and Brockes, 2012). In this frontier, Mahmoud et al. (2015) reported that pharmacological inhibition of the cholinergic nerve formation in zebrafish and newborn mice reduced cardiomyocyte proliferation following injury, thus suggesting that innervation is crucial for heart regeneration (Mahmoud et al., 2015). Moreover, the mechanical inhibition of innervation (left vagotomy) decreases the cardiac regenerative response in newborn mice that could be rescued by recombinant neuregulin 1 (NRG1) and nerve growth factor (NGF) administration. In addition, they reported that the immune response and inflammatory associated genes are downregulated following denervation, which shows that denervation impairs heart regeneration through downregulating the immune response mechanism (Mahmoud et al., 2015).

3.2. Cardiogenic modulation of stem and progenitor cells

A number of studies attempted to modulate resident or nonresident progenitor and stem cells to induce new cardiomyocyte formation following myocardial infarctions. Embryonic stem cells (ESCs) demonstrate a capacity to differentiate into beating cardiomyocytes *in vitro*. Thus, ESCs are considered to be an option to use towards regenerative cardiology. However, several drawbacks related to teratoma formation following transplantation, immune reactions, and ethical concerns

made them unsuitable for use in clinical studies (Kehat et al., 2001). The recent discovery of induced pluripotent stem cells (iPSCs) provided new angles to overcome these issues and eliminated major ethical concerns (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). iPSCs have been shown to share many characteristics of ESCs and they have been successfully generated from various somatic cells (Lowry et al., 2008; Hossini et al., 2015). In addition, differentiations of iPSCs into specific cells, including cardiomyocytes, have been reported (Gai et al., 2009; Nelson et al., 2009; Zwi et al., 2009; Ieda et al., 2010). Although there are no clinical trials yet using iPSCs cells for myocardial regeneration, intense research is currently focused on the discovery of new methods for safer cellular reprogramming methods and induction of lineage specific differentiation of iPSCs to prevent teratoma formation.

Skeletal myoblasts (satellite cells) are also thought to be a potential source for cardiac therapies. *In vitro* expansion of skeletal muscle cells derived from skeletal muscle biopsies favored them for autologous transplantation and their ischemia-resistant property made them among the first cells tried in cellular therapies in the treatment of cardiac failure (Koh et al., 1993). Engraftment of skeletal myoblasts into a damaged myocardium resulted in improved cardiac function (Taylor et al., 1998; Menasché, 2003) and prevented progressive ventricular dilatation (Pouly et al., 2004). Although several studies have suggested that engrafted skeletal myoblast cells do not transdifferentiate into functional cardiomyocytes (Reinecke et al., 2002) and electrophysiologically differ from resident cardiomyocytes (Murry et al., 1996; Leobon et al., 2003), clinical trials are still proceeding to test the potential of these cells in cardiac regenerative therapy.

3.2.1. Cardiac stem cells and induced cardiomyocytes

Cardiac stem cells (CSCs) are widely investigated in the treatment of cardiovascular disorders (reviewed in Bernstein and Srivastava (2012)). The discovery of c-kit⁺ CSCs followed the identification of other CSCs, including epicardial progenitors, Isl1⁺ cardiovascular progenitors, side population progenitors, Sca1⁺ progenitors, heterogeneous progenitors containing cardiospheres, and cardiac mesenchymal stem cells (Beltrami et al., 2003; Oh et al., 2003; Messina et al., 2004; Smith et al., 2007; Bu et al., 2009; Chong et al., 2011). Following identification of these progenitors, factors involved in the differentiation into cardiomyocytes started to emerge. Oxytocin treatment of Sca-1⁺-CD45⁻ cells was shown to induce differentiation into spontaneously beating cardiomyocytes (Matsuura et al., 2004). Furthermore, transplantation of these cells into a necrotic myocardium increased cardiac function (Oh et al., 2003). In another study, intramyocardial injection

of HGF-cMet and IGF-1 factors following induction of myocardial injury resulted in an increased number of cardiac resident stem cells (Linke et al., 2005). Qyan et al. (2007) reported that cardiac mesenchymal cells regulate the renewal and differentiation of cardiac *Isl1*⁺ cardiovascular progenitors via the Wnt/b-catenin pathway. In addition, chemical inhibition of glycogen synthase kinase-3 (GSK-3) resulted in a twofold increased number of cardiac *Isl1*⁺ cardiovascular progenitors. Cardiospheres, which were derived from endomyocardial biopsy specimens, also have a potential use in cardiac stem cell therapy. The existence of different progenitors and differentiated cells within cardiospheres mimic the stem cell niche existing in the heart, thus taking a step further in cardiac stem cell studies (reviewed in Leri et al. (2011)).

A recent study reported the direct reprogramming of cardiac fibroblasts into cardiomyocytes, which provided an alternative source of cells to trigger heart regeneration (Ieda et al., 2010). The stable integration of cardiac specific markers *Gata4*, *Tbx5*, and *Mef2x* led to the transformation of 20% of cardiac fibroblasts into induced cardiomyocytes (iCMs), which have similar epigenetic states and gene expression as normal cardiomyocytes (Ieda et al., 2010; Passier and Mummery, 2010). The integration of different factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) by Efe et al. (2011) supported the reprogramming of mouse embryonic fibroblasts into beating cardiomyocytes in 11–12 days, which was shorter when compared with the results reported by Ieda et al. (2010). A decreased trend towards tumor formation and the ability to reprogram a large number of a patient's fibroblasts into cardiomyocytes made iCMs an alternative for cardiac therapies. Even though there are many advantages of iCMs, it requires further investigations to effectively use endogenous fibroblast cells in the repair of a damaged myocardium before proceeding into clinical trials.

3.2.2. Bone marrow derived stem cells in myocardial regeneration

The bone marrow contains heterogenous cell populations. Investigations on bone marrow cells (BMCs) and hematopoietic stem cells (HSCs) in the induction of myocardial regeneration date back to the early 2000s. Orlic et al. (2001) showed that bone marrow derived cells acquire a cardiomyocyte-like phenotype and provide a functional recovery following myocardial infarction. Further clinical studies with BMCs demonstrated the prospect of human heart regeneration (Orlic et al., 2001; Strauer et al., 2002; Perin et al., 2003). Studies based on bone marrow derived MSCs indicated the ability of MSCs to induce proliferation and differentiation of resident cardiac stem cells (Chen et al., 2004; Hatzistergos et al.,

2010). Many different mechanisms have been proposed to explain the effect of BMCs in myocardial regeneration (Matsuura et al., 2004; Kajstura et al., 2005). A recent study showed that bone marrow *c-kit*⁺ cells, but not MSCs, stimulate an endogenous pool of cardiac progenitors that dilute the pool of cardiomyocyte specific GFP expression, thus improving cardiac function (Loffredo et al., 2011). In addition, the induction of a number of growth factors, including hepatocyte growth factor (HGF), insulin-like growth factor (IGF-1), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF), was reported immediately following treatment with MSCs or multipotent human BM stem cells (hBMSCs) (Yoon et al., 2005). In another study, TGF- β and bone morphogenetic protein (BMP)-2 increased the expression of cardiac transcription factors in a paracrine manner. In addition, it was reported that periostin and neuroglin administration induce cardiomyocyte proliferation. However, the source of these ligand receptor interactions (existing in both cardiomyocytes and HSCs) remains undetermined. The paracrine effect could be the underlying mechanism giving rise to a modestly improved diastolic function following BMC derived stem cell treatment.

4. Conclusion

Recent studies in different animal models of cardiac injury shed light on the underlying mechanisms and prospects of heart regeneration. The presence of barriers to rejuvenate lost cardiomyocytes such as high levels of cell cycle inhibitors and fibrosis, and the lack of factors to stimulate cardiomyocyte proliferation and stem cell differentiation into cardiac cells following myocardial injuries, are among the major issues. Thus, future studies should not only eliminate mechanistic and intrinsic molecular barriers, but also provide signals, either systematically or locally, by tissue engineering to achieve cardiac regeneration.

Acknowledgments

We apologize to colleagues whose work could not be cited and discussed because of space limitations. We thank Dr Andrew J Harvey from the Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, İstanbul, Turkey, for his critical reading of the manuscript. We are thankful for the support from the Cofunded Brain Circulation Scheme by the Scientific and Technological Research Council of Turkey (TÜBİTAK) and the Marie Curie Action COFUND of the 7th Framework Programme (FP7) of the European Commission Grant # 115C039, TÜBİTAK 1001 Grant # 115S185, the Science Academy Young Scientist Award Program (BAGEP-2015, Turkey), and funds provided by Yeditepe University in İstanbul, Turkey.

References

- Agah R, Kirshenbaum LA, Abdellatif M, Truong LD, Chakraborty S, Michael LH, Schneider MD (1997). Adenoviral delivery of E2F-1 directs cell cycle reentry and p53-independent apoptosis in postmitotic adult myocardium in vivo. *J Clin Invest* 100: 2722–2728.
- Bader D, Oberpriller J (1979). Autoradiographic and electron microscopic studies of minced cardiac muscle regeneration in the adult newt, *Notophthalmus viridescens*. *J Exp Zool* 208: 177–193.
- Becker CG, Becker T (2008). Adult zebrafish as a model for successful central nervous system regeneration. *Restor Neurol Neurosci* 26: 71–80.
- Beigi F, Schmeckpeper J, Pow-Anpongkul P, Payne JA, Zhang L, Zhang Z, Huang J, Mirotsov M, Dzau VJ (2013). C3orf58, a novel paracrine protein, stimulates cardiomyocyte cell-cycle progression through the PI3K-AKT-CDK7 pathway. *Circ Res* 113: 372–380.
- Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K et al. (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114: 763–776.
- Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H et al. (2009). Evidence for cardiomyocyte renewal in humans. *Science* 324: 98–102.
- Bergmann O, Zdunek S, Frisén J, Bernard S (2012). Cardiomyocyte renewal in humans. *Circ Res* 324: 98–102.
- Bergmann O, Zdunek S, Frisén J, Bernard S, Druid H, Jovinge S (2012). Cardiomyocyte renewal in humans. *Circ Res* 110: e17–e18.
- Bernstein HS, Srivastava D (2012). Stem cell therapy for cardiac disease. *Pediatr Res* 71: 491–499.
- Bersell K, Arab S, Haring B, Kühn B (2009). Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell* 138: 257–270.
- Bicknell Katrina A, Coxon Carmen H, Brooks G (2004). Forced expression of the cyclin B1–CDC2 complex induces proliferation in adult rat cardiomyocytes. *Biochem J* 382: 411–416.
- Borchardt T, Braun T (2007). Cardiovascular regeneration in non-mammalian model systems: what are the differences between newts and man? *Thromb Haemost* 98: 311–318.
- Bu L, Jiang X, Martin-Puig S, Caron L, Zhu S, Shao Y, Roberts DJ, Huang PL, Domian IJ, Chien KR (2009). Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. *Nature* 460: 113–117.
- Campa VM, Gutierrez-Lanza R, Cerignoli F, Diaz-Trelles R, Nelson B, Tsuji T, Barcova M, Jiang W, Mercola M (2008). Notch activates cell cycle reentry and progression in quiescent cardiomyocytes. *J Cell Biol* 183: 129–141.
- Canseco DC, Kimura W, Garg S, Mukherjee S, Bhattacharya S, Abdisalaam S, Das S, Asaithamby A, Mammen PP, Sadek HA (2015). Human ventricular unloading induces cardiomyocyte proliferation. *J Am Coll Cardiol* 65: 892–900.
- Caspi O, Huber I, Kehat I, Habib M, Arbel G, Gepstein A, Yankelson L, Aronson D, Beyar R, Gepstein L (2007). Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol* 50: 1884–1893.
- Chablais F, Veit J, Rainer G, Jaźwińska A (2011). The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. *Bmc Dev Biol* 11: 21.
- Chaudhry HW, Dashoush NH, Tang H, Zhang L, Wang X, Wu EX, Wolgemuth DJ (2004). Cyclin A2 mediates cardiomyocyte mitosis in the postmitotic myocardium. *J Biol Chem* 279: 35858–35866.
- Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S et al. (2004). Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 94: 92–95.
- Cheng RK, Asai T, Tang H, Dashoush NH, Kara RJ, Costa KD, Naka Y, Wu EX, Wolgemuth DJ, Chaudhry HW (2007). Cyclin A2 induces cardiac regeneration after myocardial infarction and prevents heart failure. *Circ Res* 100: 1741–1748.
- Chong JJ, Chandrakanthan V, Xaymardan M, Asli NS, Li J, Ahmed I, Heffernan C, Menon MK, Scarlett CJ, Rashidianfar A et al. (2011). Adult cardiac-resident MSC-like stem cells with a proepicardial origin. *Cell Stem Cell* 9: 527–540.
- D’Uva G, Aharonov A, Lauriola M, Kain D, Yahalom-Ronen Y, Carvalho S, Weisinger K, Bassat E, Rajchman D, Yifa O et al. (2015). ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. *Nat Cell Biol* 5: 627–638.
- Darehzereshki A, Rubin N, Gamba L, Kim J (2014). Differential regenerative capacity of neonatal mouse hearts after cryoinjury. *Dev Biol* 399: 91–99.
- Di Stefano V, Giacca M, Capogrossi MC, Crescenzi M, Martelli F (2011). Knockdown of cyclin-dependent kinase inhibitors induces cardiomyocyte re-entry in the cell cycle. *J Biol Chem* 286: 8644–8654.
- Efe JA, Hilcove S, Kim J, Zhou H, Ouyang K, Wang G, Chen J, Ding S (2011). Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. *Nat Cell Biol* 13: 215–222.
- Engel FB, Hsieh PCH, Lee RT (2006). FGF1/p38 MAP kinase inhibitor therapy induces cardiomyocyte mitosis, reduces scarring, and rescues function after myocardial infarction. *P Natl Acad Sci USA* 103: 5546–5551.
- Evans-Anderson HJ, Alfieri CM, Yutzey KE (2008). Regulation of cardiomyocyte proliferation and myocardial growth during development by FOXO transcription factors. *Circ Res* 102: 686–694.

- Fishman MC, Olson EN (1997). Parsing the heart: genetic modules for organ assembly. *Cell* 91: 153–156.
- Flink IL (2002). Cell cycle reentry of ventricular and atrial cardiomyocytes and cells within the epicardium following amputation of the ventricular apex in the axolotl, *Amblystoma mexicanum*: confocal microscopic immunofluorescent image analysis of bromodeoxyuridine-label. *Anat Embryol* 205: 235–244.
- Gai H, Leung EL, Costantino PD, Aguila JR, Nguyen DM, Fink LM, Ward DC, Ma Y (2009). Generation and characterization of functional cardiomyocytes using induced pluripotent stem cells derived from human fibroblasts. *Cell Biol Int* 33: 1184–1193.
- Gassmann M, Casagrande F, Orioli D, Simon H, Lai C, Klein R, Lemke G (1995). Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* 378: 390–394.
- Gemberling M, Karra R, Dickson AL, Poss KD (2015). Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. *eLife* 4: e05871.
- González-Rosa JM, Martín V, Peralta M, Torres M, Mercader N (2011). Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish. *Development* 138: 1663–1674.
- Halder G, Johnson RL (2011). Hippo signaling: growth control and beyond. *Development* 138: 9–22.
- Hatzistergos KE, Quevedo H, Oskoueï BN, Hu Q, Feigenbaum GS, Margitich IS, Mazhari R, Boyle AJ, Zambrano JP, Rodriguez JE et al. (2010). Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res* 107: 913–922.
- Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysiak E, Johnson RL, Martin JF (2011). Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science* 332: 458–461.
- Hosoda T, D'Amario D, Cabral-Da-Silva MC, Zheng H, Padin-Iruegas ME, Ogorek B, Ferreira-Martins J, Yasuzawa-Amano S, Amano K, Ide-Iwata N et al. (2009). Clonality of mouse and human cardiomyogenesis in vivo. *P Natl Acad Sci USA* 106: 17169–17174.
- Hossini AM, Megges M, Prigione A, Lichtner B, Toliat MR, Wruck W, Schröter F, Nuernberg P, Kroll H, Makrantonaki E et al. (2015). Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. *BMC Genomics* 16: 84.
- Ieda M, Fu JDD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 142: 375–386.
- Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 142: 375–386.
- Jackson T, Allard MF, Sreenan CM (1990). The c-myc proto-oncogene regulates cardiac development in transgenic mice. *Mol Cell Biol* 10: 3709–3716.
- Jessup M, Abraham WT, Casey DE, Feldman AM, Francis GS, Ganiats TG, Konstam MA, Mancini DM, Rahko PS, Silver MA et al. (2009). 2009 focused update: ACCF/AHA guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. *Circ Res* 119: 1977–2016.
- Jessup M, Brozena S (2003). Heart failure. *N Engl J Med* 348: 2007–2018.
- Jopling C, Sleep E, Raya M, Martí M, Raya A, Belmonte JCI (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* 464: 606–609.
- Jung J, Kim TG, Lyons GE, Kim HR, Lee Y (2005). Jumonji regulates cardiomyocyte proliferation via interaction with retinoblastoma protein. *J Biol Chem* 280: 30916–30923.
- Kajstura J, Rota M, Whang B, Cascapera S, Hosoda T, Bearzi C, Nurzynska D, Kasahara H, Zias E, Bonafé M et al. (2005). Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. *Circ Res* 96: 127–137.
- Kajstura J, Urbanek K, Perl S, Hosoda T, Zheng H, Ogórek B, Ferreira-Martins J, Goichberg P, Rondon-Clavo C, Sanada F, D'Amario D et al. (2010). Cardiomyogenesis in the adult human heart. *Circ Res* 107: 305–315.
- Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 108: 407–414.
- Kikuchi K, Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF, Evans T, Macrae CA, Stainier DY, Poss KD (2010). Primary contribution to zebrafish heart regeneration by *gata4*(+) cardiomyocytes. *Nature* 464: 601–605.
- Kubin T, Pöling J, Kostin S, Gajawada P, Hein S, Rees W, Wietelmann A, Tanaka M, Lörchner H, Schimanski S et al. (2011). Oncostatin M is a major mediator of cardiomyocyte dedifferentiation and remodeling. *Cell Stem Cell* 9: 420–432.
- Kühn B, Del Monte F, Hajar RJ, Chang YS, Lebeche D, Arab S, Keating MT (2007). Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. *Nat Med* 13: 962–969.
- Kumar A, Brockes JP (2012). Nerve dependence in tissue, organ, and appendage regeneration. *Trends Neurosci* 35: 691–699.
- Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassani-pour M, Police S et al. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 25: 1015–1024.
- Laflamme MA, Murry CE (2011). Heart regeneration. *Nature* 473: 326–335.

- Laflamme MA, Myerson D, Saffitz JE, Murry CE (2002). Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. *Circ Res* 90: 634–640.
- Lai D, Liu X, Forrai A, Wolstein O, Michalick J, Ahmed I, Garratt AN, Birchmeier C, Zhou M, Hartley L et al. (2010). Neuregulin 1 sustains the gene regulatory network in both trabecular and nontrabecular myocardium. *Circ Res* 107: 715–727.
- Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C (1995). Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* 378: 394–398.
- Leobon B, Garcin I, Menasche P, Vilquin JTT, Audinat E, Charpak S (2003). Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *P Natl Acad Sci USA* 100: 7808–7811.
- Lepilina A, Coon AN, Kikuchi K, Holdway JE, Roberts RW, Burns CG, Poss KD (2006). A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell* 127: 607–619.
- Leri A, Kajstura J, Anversa P (2011). Role of cardiac stem cells in cardiac pathophysiology: a paradigm shift in human myocardial biology. *Circ Res* 109: 941–961.
- Li F, Wang X, Capasso JM, Gerdes AM (1996). Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. *J Mol Cell Cardiol* 28: 1737–1746.
- Li RK, Jia ZQ, Weisel RD, Mickle DA, Zhang J, Mohabber MK, Rao V, Ivanov J (1996). Cardiomyocyte transplantation improves heart function. *Ann Thorac Surg* 62: 654–661.
- Linke A, Müller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Böhm M, Quaini F et al. (2005). Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *P Natl Acad Sci USA* 102: 8966–8971.
- Liu J, Bressan M, Hassel D, Huisken J, Staudt D, Kikuchi K, Poss KD, Mikawa T, Stainier DY (2010). A dual role for ErbB2 signaling in cardiac trabeculation. *Development* 137: 3867–3875.
- Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, Olson EN (2008). MicroRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev* 22: 3242–3254.
- Liu Y, Kitsis RN (1996). Induction of DNA synthesis and apoptosis in cardiac myocytes by E1A oncoprotein. *J Cell Biol* 133: 325–334.
- Loffredo FS, Steinhauser ML, Gannon J, Lee RT (2011). Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. *Cell Stem Cell* 8: 389–398.
- Lowry WE, Richter L, Yachechko R, Pyle AD, Tchiew J, Sridharan R, Clark AT, Plath K (2008). Generation of human induced pluripotent stem cells from dermal fibroblasts. *P Natl Acad Sci USA* 105: 2883–2888.
- Mahmoud AI, Kocabas F, Muralidhar SA, Kimura W, Koura AS, Thet S, Porrello ER, Sadek HA (2013). Meis1 regulates postnatal cardiomyocyte cell cycle arrest. *Nature* 497: 249–253.
- Mahmoud AI, O'Meara CC, Gemberling M, Zhao L, Bryant DM, Zheng R, Gannon JB, Cai L, Choi WY, Egnaczyk GF et al. (2015). Nerves regulate cardiomyocyte proliferation and heart regeneration. *Dev Cell* 34: 387–399.
- Mahmoud AI, Porrello ER, Kimura W, Olson EN, Sadek HA (2014). Surgical models for cardiac regeneration in neonatal mice. *Nat Protoc* 9: 305–311.
- Malliaras K, Zhang Y, Seinfeld J, Galang G, Tseliou E, Cheng K, Sun B, Aminzadeh M, Marbán E (2013). Cardiomyocyte proliferation and progenitor cell recruitment underlie therapeutic regeneration after myocardial infarction in the adult mouse heart. *EMBO Mol Med* 5: 191–209.
- Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T et al. (2004). Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem* 279: 11384–11391.
- Matsuura K, Wada H, Nagai T, Iijima Y, Minamino T, Sano M, Akazawa H, Molkenin JD, Kasanuki H, Komuro I (2004). Cardiomyocytes fuse with surrounding noncardiomyocytes and reenter the cell cycle. *J Cell Biol* 167: 351–363.
- Menasché P (2003). Skeletal muscle satellite cell transplantation. *Cardiovasc Res* 58: 351–357.
- Mercer SE, Odelberg SJ, Simon HGG (2013). A dynamic spatiotemporal extracellular matrix facilitates epicardial-mediated vertebrate heart regeneration. *Dev Biol* 382: 457–469.
- Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MV, Coletta M et al. (2004). Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res* 95: 911–921.
- Molkenin JD, Lin Q, Duncan SA, Olson EN (1997). Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. *Genes Dev* 11: 1061–1072.
- Mollova M, Bersell K, Walsh S, Savla J, Das LT, Park SYY, Silberstein LE, Dos Remedios CG, Graham D, Colan S et al. (2013). Cardiomyocyte proliferation contributes to heart growth in young humans. *P Natl Acad Sci USA* 110:1446–1451.
- Murry CE, Wiseman RW, Schwartz SM, Hauschka SD (1996). Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest* 98: 2512–2523.
- Neff AW, Dent AE, Armstrong JB (1996). Heart development and regeneration in urodeles. *Int J Dev Biol* 40: 719–725.
- Nelson TJ, Martinez-Fernandez A, Yamada S, Perez-Terzic C, Ikeda Y, Terzic A (2009). Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation* 120: 408–416.
- Novoyatleva T, Diehl F, Amerongen MJv, Patra C, Ferrazzi F, Bellazzi R, Engel FB (2010). TWEAK is a positive regulator of cardiomyocyte proliferation. *Cardiovasc Res* 85: 681–690.
- O'Meara CC, Wamstad JA, Gladstone RA, Fomovsky GM, Butty VL, Shrikumar A, Gannon JB, Boyer LA, Lee RT (2015). Transcriptional reversion of cardiac myocyte fate during mammalian cardiac regeneration. *Circ Res* 116: 804–815.

- Oberpriller JO, Oberpriller JC (1974). Response of the adult newt ventricle to injury. *J Exp Zool* 187: 249–253.
- Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ et al. (2003). Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *P Natl Acad Sci USA* 100: 12313–12318.
- Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P (2001). Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann NY Acad Sci* 938: 221–230.
- Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P (2003). Bone marrow stem cells regenerate infarcted myocardium. *Pediatr Transplant* 7-Suppl 3: 86–88.
- Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM et al. (2001). Bone marrow cells regenerate infarcted myocardium. *Nature* 410: 701–705.
- Passier R, Mummery C (2010). Getting to the heart of the matter: direct reprogramming to cardiomyocytes. *Cell Stem Cell* 7: 139–141.
- Pasumarthi KBS, Nakajima H, Nakajima HO, Soonpaa MH, Field LJ (2005). Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. *Circ Res* 96: 110–118.
- Pelster B, Burggren WW (1996). Disruption of hemoglobin oxygen transport does not impact oxygen-dependent physiological processes in developing embryos of zebra fish (*Danio rerio*). *Circ Res* 79: 358–362.
- Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, Rossi MI, Carvalho AC, Dutra HS, Dohmann HJ et al. (2003). Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 107: 2294–2302.
- Piatkowski T, Mühlfeld C, Borchardt T, Braun T (2013). Reconstitution of the myocardium in regenerating newt hearts is preceded by transient deposition of extracellular matrix components. *Stem Cells Dev* 22: 1921–1931.
- Poolman RA, Li JM, Durand B, Brooks G (1999). Altered expression of cell cycle proteins and prolonged duration of cardiac myocyte hyperplasia in p27KIP1 knockout mice. *Circ Res* 85: 117–127.
- Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA (2011). Transient regenerative potential of the neonatal mouse heart. *Science* 331: 1078–1080.
- Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D, Mammen PP, Rothermel BA, Olson EN, Sadek HA (2013). Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. *P Natl Acad Sci USA* 110: 187–192.
- Poss KD, Wilson LG, Keating MT (2002). Heart regeneration in zebrafish. *Science* 298: 2188–2190.
- Pouly J, Hagège AA, Vilquin JTT, Bissery A, Rouche A, Bruneval P, Duboc D, Desnos M, Fiszman M, Fromes Y et al. (2004). Does the functional efficacy of skeletal myoblast transplantation extend to nonischemic cardiomyopathy? *Circulation* 110: 1626–1631.
- Pu WT, Ishiwata T, Juraszek AL, Ma Q, Izumo S (2004). GATA4 is a dosage-sensitive regulator of cardiac morphogenesis. *Dev Biol* 275: 235–244.
- Puente BN, Kimura W, Muralidhar SA, Moon J, Amatruda JF, Phelps KL, Grinsfelder D, Rothermel BA, Chen R, Garcia JA et al. (2014). The oxygen-rich postnatal environment induces cardiomyocyte cell-cycle arrest through DNA damage response. *Cell* 157: 565–579.
- Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P (2002). Chimerism of the transplanted heart. *N Engl J Med* 346: 5–15.
- Qyang Y, Martin-Puig S, Chiravuri M, Chen S, Xu H, Bu L, Jiang X, Lin L, Granger A, Moretti A et al. (2007). The renewal and differentiation of Isl1+ cardiovascular progenitors are controlled by a Wnt/ β -catenin pathway. *Cell Stem Cell* 1: 165–179.
- Raya A, Koth CM, Büscher D, Kawakami Y, Itoh T, Raya RM, Sternik G, Tsai HJJ, Rodríguez-Esteban C, Izpisua-Belmonte JC (2003). Activation of notch signaling pathway precedes heart regeneration in zebrafish. *P Natl Acad Sci USA* 100 Suppl: 11889–11895.
- Reinecke H, Poppa V, Murry CE (2002). Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol* 34: 241–249.
- Rochais F, Sturny R, Chao CM (2014). FGF10 promotes regional foetal cardiomyocyte proliferation and adult cardiomyocyte cell-cycle re-entry. *Cardiovasc Res* 104: 432–442.
- Rumyantsev PP (1966). Autoradiographic study on the synthesis of DNA, RNA, and proteins in normal cardiac muscle cells and those changed by experimental injury. *Folia Histochem Cytochem* 4: 397–424.
- Rumyantsev PP (1973). Post-injury DNA synthesis, mitosis and ultrastructural reorganization of adult frog cardiac myocytes. An electron microscopic-autoradiographic study. *Z Zellforsch Mik Ana* 139: 431–450.
- Sander V, Davidson AJ (2014). Kidney injury and regeneration in zebrafish. *Sem Nephrol* 34: 437–444.
- Schnabel K, Wu CC, Kurth T, Weidinger G (2011). Regeneration of cryoinjury induced necrotic heart lesions in zebrafish is associated with epicardial activation and cardiomyocyte proliferation. *PLoS One* 6: e18503.
- Senyo SE, Steinhauser ML, Pizzimenti CL, Yang VK (2013). Mammalian heart renewal by pre-existing cardiomyocytes. *Nature* 493: 433–436.
- Senyo SE, Steinhauser ML, Pizzimenti CL, Yang VK, Cai L, Wang M, Wu TDD, Guerquin-Kern JLL, Lechene CP, Lee RT (2013). Mammalian heart renewal by pre-existing cardiomyocytes. *Nature* 493: 433–436.

- Shiba Y, Fernandes S, Zhu WZ, Filice D, Muskheli V, Kim J, Palpant NJ, Gantz J, Moyes KW, Reinecke H et al. (2012). Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature* 489: 322–325.
- Singh BN, Koyano-Nakagawa N, Garry JP, Weaver CV (2010). Heart of newt: a recipe for regeneration. *J Cardiovasc Transl Res* 3: 397–409.
- Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR, Marban E (2007). Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation* 115: 896–908.
- Soonpaa MH, Field LJ (1997). Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. *Am J Physiol* 272: H220–H226.
- Soonpaa MH, Kim KK, Pajak L, Franklin M, Field LJ (1996). Cardiomyocyte DNA synthesis and binucleation during murine development. *Am J Physiol* 271: H2183–H2189.
- Soonpaa MH, Koh GY, Pajak L, Jing S, Wang H, Franklin MT, Kim KK, Field LJ (1997). Cyclin D1 overexpression promotes cardiomyocyte DNA synthesis and multinucleation in transgenic mice. *J Clin Invest* 99: 2644–2654.
- Strauer BE, Brehm M, Zeus T, Köstering M, Hernandez A, Sorg RV, Kögler G, Wernet P (2002). Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106: 1913–1918.
- Sulima VI (1968). O regeneratsii miokarda reptilii pri razlichnykh povrezhdeniiakh stenki serdtsa. *Arkh Anat Gistol Embriol* 55: 56–63 (in Russian).
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861–872.
- Takahashi K, Yamanaka S (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663–676.
- Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE (1998). Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 4: 929–933.
- Tseng AS, Engel FB, Keating MT (2006). The GSK-3 inhibitor BIO promotes proliferation in mammalian cardiomyocytes. *Chem Biol* 13: 957–963.
- von Gise A, Lin Z, Schlegelmilch K, Honor LB, Pan GM, Buck JN, Ma Q, Ishiwata T, Zhou B, Camargo FD et al. (2012). YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. *P Natl Acad Sci USA* 109: 2394–2399.
- Walsh S, Pontén A, Fleischmann BK, Jovinge S (2010). Cardiomyocyte cell cycle control and growth estimation in vivo—an analysis based on cardiomyocyte nuclei. *Cardiovasc Res* 86: 365–373.
- Wang J, Panáková D, Kikuchi K, Holdway JE (2011). The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. *Development* 138: 3421–3430.
- Wills AA, Holdway JE, Major RJ, Poss KD (2008). Regulated addition of new myocardial and epicardial cells fosters homeostatic cardiac growth and maintenance in adult zebrafish. *Development* 135: 183–192.
- Witman N, Murtuza B, Davis B, Arner A, Morrison JI (2011). Recapitulation of developmental cardiogenesis governs the morphological and functional regeneration of adult newt hearts following injury. *Dev Biol* 354: 67–76.
- Xiao F, Kimura W, Sadek HA (2015). A hippo “AKT” regulates cardiomyocyte proliferation. *Circ Res* 116: 3–5.
- Xin M, Kim Y, Sutherland LB, Qi X, McAnally J, Schwartz RJ, Richardson JA, Bassel-Duby R, Olson EN (2011). Regulation of insulin-like growth factor signaling by Yap governs cardiomyocyte proliferation and embryonic heart size. *Sci Signal* 4: ra70.
- Xin M, Olson EN, Bassel-Duby R (2013). Mending broken hearts: cardiac development as a basis for adult heart regeneration and repair. *Nat Rev Mol Cell Bio* 14: 529–541.
- Yeh ET, Zhang S, Wu HD, Körbling M, Willerson JT, Estrov Z (2003). Transdifferentiation of human peripheral blood CD34+ enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation* 108: 2070–2073.
- Zeisberg EM, Ma Q, Juraszek AL, Moses K, Schwartz RJ, Izumo S, Pu WT (2005). Morphogenesis of the right ventricle requires myocardial expression of Gata4. *J Clin Invest* 115: 1522–1531.
- Zwi L, Caspi O, Arbel G, Huber I, Gepstein A, Park IH, Gepstein L (2009). Cardiomyocyte differentiation of human induced pluripotent stem cells. *Circulation* 120: 1513–1523.