Underlying mechanisms and prospects of heart regeneration

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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 therapeutic 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...
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.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Zebrafish</th>
<th>Newt</th>
<th>Neonatal Mice</th>
<th>Adult Mice</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury or treatment</td>
<td>Apical resection</td>
<td>Apical resection</td>
<td>Apical resection (7 day old mouse)</td>
<td>-</td>
<td>Use of LVAD</td>
</tr>
<tr>
<td>Cryoinjury</td>
<td>Basal resection</td>
<td>Cryoinjury</td>
<td>Cryoinjury</td>
<td>Ischemic MI</td>
<td>Ischemic MI</td>
</tr>
<tr>
<td>Genetic ablation</td>
<td>-</td>
<td>Ischemic MI</td>
<td>Ischemic MI</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Regeneration and time</td>
<td>Regeneration in 60 days</td>
<td>Regeneration in 21 days</td>
<td>Fibrosis &amp; no regeneration</td>
<td>Fibrosis &amp; no regeneration (modest CM proliferation)</td>
<td></td>
</tr>
<tr>
<td>Regeneration in 30–45 days</td>
<td>Regeneration in 21 days</td>
<td>Fibrosis &amp; no regeneration</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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) (Wittman 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 (cmclc2). 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 tenasin-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 $^{14}$C 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; Lalflamme 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 multisotope 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 (Lalflamme 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...
contractile proteins and the induction of DNA synthesis without cytokinesis, resulting in binucleation and cell cycle arrest at G<sub>0</sub>/G<sub>1</sub> (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 precooled 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.
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<sup>Waf1</sup>, p27<sup>Kip1</sup>, and p57<sup>Kip2</sup>) 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; Kühn 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).

Table 3. Major factors involved in cardiomyocyte proliferation.

<table>
<thead>
<tr>
<th>Cardiogenic factors and manipulations</th>
<th>Fold change in proliferating cardiomyocytes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meis1 knockout</td>
<td>9 (pH3 + CMs, Aurora B + CMs)</td>
<td>(Mahmoud et al., 2013)</td>
</tr>
<tr>
<td>GSK-3 inhibition (BIO)</td>
<td>5 (pH3 + CMs)</td>
<td>(Tseng et al., 2006)</td>
</tr>
<tr>
<td>Foxo1 dominant negative</td>
<td>2.5 (pH3 + CMs)</td>
<td>(Evans-Anderson et al., 2008)</td>
</tr>
<tr>
<td>miR-133a knockout</td>
<td>2.3 (pH3 + CMs)</td>
<td>(Liu et al., 2008)</td>
</tr>
<tr>
<td>Junonji knockout</td>
<td>2.2 (pH3 + CMs)</td>
<td>(Jung et al., 2005)</td>
</tr>
<tr>
<td>p27&lt;sup&gt;Kip1&lt;/sup&gt; knockout</td>
<td>2-3 (troponin I + CMs)</td>
<td>(Poolman et al., 1999)</td>
</tr>
<tr>
<td>Constitutively active ERBB2</td>
<td>&gt;12 (Ki67 + CMs, pH3 + CMs, Aurora B+ CMs)</td>
<td>(D’Uva et al., 2015)</td>
</tr>
<tr>
<td>Nrg1 (or FGF1, periostin) treatment</td>
<td>&gt;4 (BrdU + CMs, Aurora B + CMs, pH3 + CMs,)</td>
<td>(Bersell et al., 2009)</td>
</tr>
<tr>
<td>Activated Yap1</td>
<td>&gt;7 (Ki67 + CMs, pH3 + CMs, Aurora B+ CMs)</td>
<td>(von Gise et al., 2012)</td>
</tr>
<tr>
<td>Salv knockout</td>
<td>&gt;4 (pH3 + CMs)</td>
<td>(Heallen et al., 2011)</td>
</tr>
<tr>
<td>IL13 (or IL3, CTGF, Nrg1) treatment</td>
<td>&gt;1.5 (3H Thymidine CMs, Ki67 + CMs, BrdU + CMs)</td>
<td>(O’Meara et al., 2015)</td>
</tr>
<tr>
<td>Oncostatin M treatment</td>
<td>&gt;2 (EdU + CMs)</td>
<td>(Kubin et al., 2011)</td>
</tr>
<tr>
<td>TWEAK treatment</td>
<td>6.2 (BrdU + CMs)</td>
<td>(Novoyaytseva et al., 2010)</td>
</tr>
<tr>
<td>C3orf58 treatment</td>
<td>&gt;2 (Ki67 + CMs, BrdU + CMs, Aurora B + CMs)</td>
<td>(Beigi et al., 2013)</td>
</tr>
<tr>
<td>Periostin treatment</td>
<td>&gt;5 (BrdU + CMs, pH3 + CMs, Aurora B+ CMs)</td>
<td>(Kühn et al., 2007)</td>
</tr>
<tr>
<td>FGF10 treatment</td>
<td>2 (Ki67 + CMs, pH3 + CMs,)</td>
<td>(Rochais et al., 2014)</td>
</tr>
<tr>
<td>Cyclin D2 overexpression</td>
<td>&gt;5 (MHC-nLAC + CMs)</td>
<td>(Pasumarthi et al., 2005)</td>
</tr>
<tr>
<td>Cyclin B1-CDC2 or cyclin a2 overexpression</td>
<td>&gt;1.4 (CMs)</td>
<td>(Bicknell et al., 2004)</td>
</tr>
<tr>
<td>Activated notch</td>
<td>&gt;7 (Ki67 + CMs, BrdU + CMs, Aurora B + CMs)</td>
<td>(Campa et al., 2008)</td>
</tr>
<tr>
<td>c-myc or E1A overexpression</td>
<td>2 (CMs)</td>
<td>(Jackson et al., 1990)</td>
</tr>
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</table>

CM: cardiomyocytes.
Signal 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 (Falke 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 I (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).

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., 2009; Oh et al., 2003). In another study, intramyocardial injection
of HGF-cMet and IGF-1 factors following induction of myocardial resident injury resulted in an increased number of cardiac mesenchymal cells regulating 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 making a step forward 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 Mef2C 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 undefined. 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.

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