We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



9

Are We There Yet? A Story About Cardiac Stem Cells

Shizuka Uchida, Piera De Gaspari and Thomas Braun
Max-Planck-Institute for Heart and Lung Research
Germany

1. Introduction

The heart is the first organ which becomes functional during embryonic development. During lifetime, it cannot rest or stop working without sacrificing the whole organism. Nobody would question that the heart is one of the most vital organs in the body. Severe diseases of the heart compromise the quality of life and often lead to premature death. Cardiovascular diseases are currently the number one killer around the world, and it seems likely that this will not change in the near future. It is therefore not a surprise that huge efforts are made to treat cardiac disease and more recently to repair damaged hearts. However, up to now, there is no causative and definitive cure for cardiovascular diseases although major therapeutic improvements were made. Yet, most therapeutic approaches tackle symptoms and not causes, which is in part due to the complex etiology of cardiovascular diseases (e.g. environmental, genetic, life style, physiological, etc. (Kullo & Cooper, 2010)).

During the last halve of the century, the view predominated that the heart is essentially a post-mitotic organ, overthrowing previous assumptions, which reasoned that the heart is able to regenerate. The latter theory persisted from 1850 to the first quarter of the 20th century and was then replaced by the belief that remodelling events of the heart are solely based on growth rather than on proliferation of cardiomyocytes (Carvalho & de Carvalho, 2010; Karsner et al., 1925). More recently, the view that cardiomyocytes cannot renew has been put into question. Piero Anversa's group published a series of articles claiming that ongoing cell death of cardiomyocytes in the heart requires extensive replacement of cardiomyocytes. It is not surprising that this hypothesis stirred a lot of controversy (Anversa et al., 2007). One should not forget, however, that the concept of cardiomyocyte renewal is not completely new. A very low but nevertheless detectable level of cardiomyocyte cell cycle activity was reported in rodent studies almost 50 years ago (Soonpaa & Field, 1998).

One of the major challenges in the field has been the detection of newly formed cardiomyocytes and/or the visualization of cell division events. This problem has been, at least partially, overcome by utilizing clever labelling schemes, which rely on the intentional or non-intentional labelling of the genetic material of cardiomyocytes. A study by Jonas Frisén and his colleagues (Bergmann et al., 2009) took advantage of the nuclear fall-out that was generated during nuclear bomb testing until 1963. The carbon-14 (C14) isotope that was released into the atmosphere was incorporated into plants and animals or diffused into the oceans to enter the food cycle and eventually "labelled" human beings. Using the C14

concentration in cardiomyocyte genomic DNA, Frisén's group calculated the age of cardiomyocytes. The authors estimated that human cardiomyocytes renew at a rate of 1% per year at the age of 20, which declines to 0.45% at the age of 75. According to their calculation, fewer than 50% of cardiomyocytes are renewed during a normal life span of a human being. In 2010, Anversa's group reported a much higher rate of turnover using materials from cancer patients who received infusion of the radiosensitizer iododeoxyuridine (IdU) for therapeutic purposes. IdU is rapidly incorporated into cycling cells thereby setting up a pulse-and-chase "experiment" (similar to bromodeoxyuridine (BrdU)-labelling (DuFrain et al., 1984)). According to the IdU study, the turnover rate of cardiomyocytes is 22% per year (20% and 13% for fibroblasts and endothelial cells, respectively). The lifespan of cardiomyocytes was calculated to be ~4.5 years resulting in the claim that cardiomyocytes renew several times during a normal life span (Kajstura et al, 2010a). To further support their hypothesis, Anversa's group published another report (Kajstura et al, 2010b), which demonstrated that the ageing human heart of women is more adoptive to cardiomyocyte loss than that of men (Olivetti et al., 1995). By counting cardiomyocytes and c-kit+ cardiac stem cells (CSCs) undergoing apoptosis, cellular senescence and proliferation, the authors concluded that from 20 to 100 years of age, the entire cardiomyocyte compartment of women is replaced 15 times, whereas that of men is renewed 11 times. These numbers, which are provocatively high, are awaiting independent confirmation from other labs.

At the first glance, results of the above mentioned 3 reports in human seem to contradict findings in mice published in 2007. By utilizing transgenic mice to trace the fate of adult cardiomyocytes based on alpha-MHC-driven GFP expression, Hsieh et al. (Hsieh et al., 2007) observed cardiomyocyte turnover by stem or precursor cells after myocardial infarction or pressure overload. However, during normal ageing up to one year (which is equivalent to a 34-year-old human (Holaska, 2008)), the authors did not record a significant turnover of cardiomyocytes. Yet, a careful analysis of the published data reveals that labelling based on tamoxifen injections is not 100% (82.7 ± 1.7% in the above study), and there is a 1-2 percent fluctuation of labelled cells, which explain differences to the results by Bergmann et al. (Bergmann et al., 2009).

Although the above mentioned studies in human cardiomyocyte turnover are intriguing and provide impressive results, some problems remain. Human cardiomyocytes initiate cell cycle activity in response to mechanical stress (e.g. hypertrophy) without nuclear or cell division (karyo- and cyto-kinesis, respectively) (Adler & Friedburg, 1986; Soonpaa & Field, 1998), which might lead to an increase of the number of labelled cardiomyocytes. Surprisingly, there is no consensus in the literature regarding the number of nuclei in human cardiomyocytes. While murine cardiomyocytes are >90% binucleated (Liu et al., 2010; Soonpaa et al., 1996), it has been reported that 74% of human cardiomyocytes are mononucleated, compared to 25.5% for bi-, 0.4% for tri- and 0.1% for tetranucleated cells (Olivetti et al., 1996). The calculations of Anversa's group in recent publications relied mostly on those numbers (Kajstura et al, 2010a, 2010b). On the other hand, Frisén's group argued that the bulk of human cardiomyocytes is not mononucleated. According to their calculations, the majority of cardiomyocyte nuclei from the human adult left ventricle has more than two complete sets of chromosomes, (i.e. 33.5, 55.8 and 10.7% are di-, tetra- and octa-ploid, respectively (Bergmann et al., 2011)). It should not be too difficult to resolve this discrepancy in the future.

Given the findings discussed above, it seems safe to state that cardiomyocytes undergo a certain degree of renewal during the lifetime of a mammalian organism. The cellular source

of cardiomyocyte renewal, however, remains an open question. In principal, it is possible that new cardiomyocytes are generated from already existing cardiomyocytes, a principle that dominates embryonic heart development. Alternatively, different types of cardiac stem cells might generate new cardiomyocytes and other cell types following an endogenous program or inductive stimuli. To address these possibilities, it is required to utilize proper animal models since the required experimental manipulation is not applicable to human beings. Therefore, we will first survey the origin of cells in the heart and then pay special attention to putative cardiac stem cells (CSC) in the murine heart.

2. Cell types in heart and their lineages

Researchers from other fields often assume that the heart is mostly made up from cardiomyocytes, which is not true. The mouse heart is composed of ~56% cardiomyocytes, 27% fibroblasts, 10% vascular smooth muscle cells and 7% endothelial cells (Banerjee et al., 2007). Most likely, several other cell types are hidden within these principal groups awaiting further characterization. Interestingly, the distribution of cell types in the heart varies between different rodent species. Rat hearts contain only 26.4% cardiomyocytes, a number that is similar to human beings. The major cell type in rat and human hearts is the fibroblast (62.6%) (Banerjee et al., 2007; Nag, 1980). If we look at the composition of cell types in the heart, rats appear to be rather similar to humans (Rubart & Field, 2006). Moreover, there are several other reasons which added to the popularity of the rat as a cardiovascular model system compared to mice (Aitman et al., 2008). However, advanced genetic manipulation of rats was not possible so far (Cui et al., 2011; Tong et al., 2010) although the advent of zinc finger nucleases (Geurts et al., 2009) and rat ES cells (Buehr et al., 2008; Li et al., 2008) might change that picture in the future. Given that the composition of cell types differs greatly between human and mice, it might not be possible to translate all findings in mice directly to humans. Nevertheless, the advanced state of mouse genetics greatly facilitates analysis of CSCs.

2.1 Cardiomyocytes

Cardiomyocytes constitute the functionally most relevant part of the myocardium, which generates the necessary force enabling the heart to act as a pump. Although the view about cardiomyocyte turnover might have changed over the years, several studies carefully documented birth and growth of cardiomyocytes. For example, Loren Field's group (Soonpaa et al., 1996) reported that DNA synthesis in cardiomyocytes occurs in two distinct phases during the murine development. The first phase is associated with cardiomoycyte proliferation, which occurs during fetal life. The second phase follows after the cession of reduplication of cardiomyocytes (transition from the first phase takes place before day 10 after birth). The feature of this phase is binucleation of cardiomyocytes due to a round of genomic duplication and karyokinesis without cytokinesis.

Currently, the following four sources of cardiomyocyte renewal are considered (Parmacek & Epstein, 2009):

1. Adult cardiomyocytes reentering the cell cycle and divide
2. Bone-marrow-derived cardiac stem/progenitor cells
3. Cells derived from the embryonic epicardium
4. Cardiac stem/progenitor cells

Since a lack of sufficient number of cardiomyocytes can cause many forms of congenital and adult cardiovascular diseases, intensive research has been conducted to find a set of genes/proteins that might drive adult cardiomyocytes into cell cycle to regenerate cardiomyocytes (Rubart & Field, 2006). However, one needs to keep in mind that such approaches bear some inherent problems and that it is absolutely required to prevent uncontrolled proliferation. Since primary cardiac tumours are very rare (Devbhandari et al., 2007), there must be an intrinsic, biological block, which prevents division of cardiomyocytes soon after birth. Studies by several laboratories revealed that it is rather difficult to overcome this block and to achieve controlled proliferation of adult cardiomyocytes (Ebelt et al., 2005, 2006, 2008a, 2008b). There is still hope, however, to utilize the remaining, low-level potential of cardiomyocytes to proliferate for therapeutic purposes.

The second acclaimed source of cardiomyocyte renewal is bone-marrow-derived mesenchymal stem cells (BMSCs). Transdifferentiation of such cells has been fiercely debated over the years, which is well beyond the scope of this chapter. Interested readers might consult the excellent articles (Alaiti et al., 2010; Phinney & Prockop, 2007; Psaltis et al., 2008), which cover this topic. The majority view in the field sees BMSCs as beneficial to treat patients suffering from acute myocardial infarction and ischemic heart failure (Chugh et al., 2009). However, transdifferentiation of transplanted or injected BMSCs (fresh or cultured) are not very likely to contribute to the success of these therapies; instead, BMSCs secrete growth factors and cytokines that might enhance survival of surviving cardiomyocytes and stimulate endogenous repair mechanisms via activation of resident CSCs and other stem cells (Wen et al., 2010). Regarding the issue of transdifferentiation, an interesting study was conducted by Nern et al. (Nern et al., 2009). In this study, the authors employed a lineage tracing system based on the haematopoietic-specific promoter vav to monitor cell fusion events under physiological conditions to challenge transplantation studies using BMSCs. In the case of the heart, the authors found only a single LacZ-positive (the reporter gene from Rosa26 LacZ allele) cardiomyocyte in four hearts of non-irradiated healthy trasgenic mice (vav-iCre/Rosa26 LacZ). Therefore, it is unlikely that haematopoietic cells contribute to renewal of cardiomyocytes. Of course, one might argue that the labelled cells are different from BMSCs but current evidence does not support the view that BMSCs act as major players for the cardiomyocyte renewal.

The third potential source of cardiomyocyte renewal is so-called "epicardially derived mesenchymal cells (EPDCs)" (Morabito et al., 2001). EPDCs are derived from a subpopulation of epicardial cells and were shown to differentiate into cardiac vessels, cardiomyocytes and connective tissue of heart (Limana et al., 2011). Some authors name these cells "cardiac stem cells" (Wessel & Pérez-Pomares, 2004) although several other cell populations, which reside within the myocardium have also been dubbed this way. A more comprehensive description of different population of putative cardiac stem cells is given below in Section 3.

2.2 Smooth muscle cells

Smooth muscle cells are highly plastic and might toggle between a contractile and synthetic phenotype in response to extracellular cues unlike their striated muscle cousins (cardiac and skeletal muscle cells) (Owens, 1995). In the heart, the tone of vascular smooth muscle cells regulates the diameter of blood vessels, blood pressure and blood flow (Rzucidlo et al., 2007), which are integral to the function of the heart. Dysregulation of smooth muscle cells promote various diseases, including atherosclerosis, which results in over 55% of all deaths

in Western civilization (Owens et al., 2004). Therefore, significant efforts have been made to elucidate pathological mechanisms affecting vascular smooth muscle cells. Vascular progenitor cells (Kumar & Caplice, 2010) have been claimed to represent a potential source for the renewal of damaged or diseased smooth muscle cells. Since the major source of such progenitor cells are from vascular walls, which consists of endothelial cells, they will be discussed in the next subsection.

2.3 Endothelial cells

In arteries and arterioles, endothelial cells are closely associated with vascular smooth muscle cells while capillaries are devoid of smooth muscle cells (Ergün, 2011). Due to their importance, endothelial cells have been studied intensively and numerous excellent reviews are available. Here, we discuss briefly potential progenitor cells of the endothelium known as "vascular progenitor cells" or "endothelial progenitor cells (EPCs)".

The first report about EPCs in 1997 (Asahara et al., 1997) gave rise to numerous other studies. Most researchers relied on surface markers to identify EPCs, sort them and put them in culture to differentiate them in vitro to test their plasticity. Such studies resulted in lab-to-lab, equipment-to-equipment and reagent-to-reagent (e.g. antibodies) differences in the results, which lead to discussions of contaminations by other cell types (Prokopi et al., 2009). However, the therapeutic potential of EPCs appears relatively high. Numerous clinical trials have been conducted, which mostly reported beneficial effects (Kumar & Caplice, 2010).

The most confusing issue about EPCs is the lack of defined marker and niches (Ergün, 2011; Psaltis et al., 2010). This lead to the claim that EPCs might belong to the haematopoietic lineage and share its niche and origin (Richardson & Yoder, 2011). The contribution of EPCs to neo-angiogenesis is controversial. Some reports describe a very strong contribution to the endothelium of different vessels whereas other studies demonstrated only a moderate if any contribution. A contribution of EPCs to parenchymal cells of the heart appears unlikely. Lineage tracing experiments using the haematopoietic-specific promoter vav did not indicate a contribution to cardiomyocytes (Nern et al., 2009) although it is possible that some EPCs are not derived from the haematopoietic lineage.

Endothelial and smooth muscle cells are relatively plastic, which might allow them to acquire different fates after de-differentiation and re-differentiation including fibroblasts (Stintzing et al., 2009; Zeisberg et al., 2007). All these considerations make it difficult to judge the contributions of such EPCs to the renewal of myocardial cell types without a lineage tracing study using a clearly defined marker for EPCs, which is currently non-existing.

2.4 Cardiac fibroblasts

Cardiac fibroblasts comprise a rather heterogenous group of cells. Due to the absence of a single pathognomonic marker for fibroblasts, virtually all interstitial cells, which are not associated with vessels and which are not cardiomyocytes, are considered fibroblasts. The unusual phenotypic plasticity of fibroblasts does also raise the question whether they represent a single mature cell type or also comprise different types of precursor cells (Eyden, 2004). This point becomes clearer when considering the morphology of primary BMSCs in culture, which are often described to have "fibroblast-like" morphology (Friedenstein et al., 1976), an attribute that is also used to describe adult stem cells in culture (Rios & Williams,1990). CSCs, for example, show a very similar morphology, which makes it difficult to distinguish them from fibroblasts without additional molecular markers (Messina et al., 2004). By definition, CSCs should give rise to all the lineages in heart,

including cardiac fibroblasts. It probably will remain a lasting challenge for some time to distinguish CSCs from fibroblasts and from intermediary cell types that have been generated from CSCs but not yet acquired a classical fibroblastic phenotype.

3. Current status of cardiac stem cells

When one speaks about CSCs, currently there are 6 schools (Table 1).

Type of CSCs	Other Markers	References
Sca1+ CSCs	CD34-, CD45-, FLK1-, c-kit+/-, GATA4+, NKX2-5+/-, MEF2C+	(Oh et al., 2003; Forte et al., 2008; Matsuura et al., 2004; Rosenblatt-Velin et al., 2005; Tateishi et al., 2007; Wang et al., 2006; Wu et al., 2006)
c-kit+ CSCs	CD34-, CD45-, Sca1+, GATA4+, NKX2-5+, MEF2C+	(Bearzi et al., 2007; Beltrami et al., 2003; Dawn et al., 2005; Linke et al., 2005; Miyamoto et al., 2010; Tillmanns et al., 2008; Urbanek et al., 2003)
Isl-1+ CSCs	CD31-, Sca1-, c-kit-, GATA4+, NKX2-5+	(Laugwitz et al., 2005; Moretti et al., 2006)
Side population (SP) cells	CD34+, CD45+, ABCG2+, Sca1+, c-kit+, NKX2-5-, GATA4-	(Martin et al., 2004; Liang et al., 2010; Oyama et al., 2007; Pfister et al., 2005)
Cardiospheres	CD34+, CD45+, ABCG2+, Sca1+, c-kit+, NKX2-5-, GATA4-	(Messina et al., 2004; Andersen et al., 2009; Cheng et al., 2010; Davis et al., 2010; Smith et al., 2007; Tateishi et al., 2007)
Cardiac mesangioblasts (EPCs)	CD31+, CD34+, CD44+, CD45-, Sca1+, c-kit+	(Barbuti et al., 2010; Galvez et al., 2008; Gálvez et al., 2009)

Table 1. List of resident CSCs

Due to the importance of heart as a vital organ, several attempts have been made to characterize CSCs using various techniques. Numerous review articles were published covering the field of CSCs (Anversa et al., 2006; Bollini et al., 2011; Di Nardo et al., 2010; Musunuru et al., 2010; Tateishi et al., 2008). Considering the acclaimed types of CSCs, it seems that heart, once considered to be a post-mitotic organ, harbours the highest number of adult stem cells in the body. At present, it is not clear whether CSCs can be called “true” adult stem cells with a “true” multipotency (Ellison et al., 2010; Stamm et al., 2009). The concept of multipotent adult stem cells and progenitor cells (sometime classified as "transient-amplifying cells") (Bryder et al., 2006) is derived from the haematopoietic system and might not necessarily apply to CSCs.

As described in previous chapters, postnatal cardiomyocytes are likely to be renewed throughout the life of a mammal. The cellular sources of postnatally emerging cardiomyocytes are probably CSCs although definitive evidence is missing. Similarly, CSCs appear to be situated within the heart and not in the bone marrow or other locations outside the heart although such a possibility has not been unequivocally ruled out. Recent findings of circulating EPCs and "very small embryonic-like stem cells (VSELs)" (Ratajczak et al., 2009) renewed the debate about the origin of CSCs although the impact of such studies

seems limited. Virtually all studies devoted to "circulating" ("non-resident") cells are based on ex vivo experiments. In such experiments, putative stem cells are isolated using antibodies against surface markers followed by transplantation into "hosts". This procedure is similar to bone marrow transplantation experiments, which sometimes results in fusion of transplanted cells to host cells or uptake of marker proteins (e.g. GFP) by host cells (Nern et al., 2009). In contrast, no clear evidence exists that intercellular fusions occur under physiological conditions in the heart involving, for example, cardiomyocytes and endothelial cells. In fact, classical genetic lineage tracing experiments and injection of genetically labelled cells into mouse blastocyts argue against widespread cellular fusion events (Schulze et al., 2005).

3.1 Lineage tracing

The "gold standard" to define the origin of a particular set of cells is a "lineage tracing" experiment using a defined and established stem cell marker gene. Long-term lineage tracing experiments rely on model organisms using transgenic technology. To understand the theoretical basis of lineage tracing, one needs to distinguish between transient and permanent cell labelling. Transient labelling is based on knock-in of a reporter gene (e.g. GFP) into a gene of interest to visualize the current activity of a specific gene. This has been successfully applied to observe the contributions of c-kit+ cells to the revascularizing infarct regions of the myocardial infarcted heart by utilizing c-kit(BAC)-EGFP mice (Tallini et al., 2009). Labelled cells in the infarct regions were not cardiomyocytes but endothelial and smooth muscle cells. Under normal physiological condition, the authors observed an increasing number of labelled cells in the heart up to postnatal day 2. Thereafter, the number declined, and the labelled cells were rarely observed in an adult heart. The authors concluded from these results that "c-kit expression in cardiomyocytes in the adult heart after injury does not identify cardiac myogenesis." (Tallini et al., 2009). As one can easily imagine, this is due to the limitation of transient labelling, since labelled cells will loose the label upon differentiation.

In contrast, a permanent labelling strategy, which directly targets the genetic material, can bypass problems that arise from changes in the transcriptional program. This can be done by utilizing mouse strains that carry a conditionally active Cre-recombinase and a Cre-dependent reporter gene (Smedley et al., 2010). Some researchers use inducible systems (e.g. tamoxifen-inducible Mer-Cre-Mer system (Petrich et al., 2003)) or double transgenic mice (Cre-reporter) and triple transgenic mice based on tet-Cre system (Tang et al., 2008).

3.2 A potential lineage tracing study to monitor contribution of CSCs to the myocardium

A permanent labelling strategy to trace the fates of adult stem cells might utilize a tetracycline transactivator (tTA) placed under the control of stem cell marker gene as shown in Fig. 1. Preferably, such a system should be based on a "tet-off" design, in which the system is activated until doxycycline (DOX) is added (Gossen & Bujard, 1992; Urlinger et al., 2000). The opposite system is called "tet-on" (rtTA is used), which is claimed to allow a tight control (Stary et al., 2010). However, when tet-on system is used, DOX must be administered in the drinking water of mice, which can be rather expensive. In addition, DOX inhibits angiogenesis in mouse (Cox et al., 2010; Fainaru et al., 2008). In our lab, the DOX-treated murine hearts (1mg/ml in the drinking water) tend to be ~30% smaller compared to the age-matched untreated hearts (data not shown). Furthermore, DOX might also have effects on the

attenuation of cardiac hypertrophy through the inhibition of matrix metalloproteases (Errami et al., 2008), which makes it difficult to challenge such mice by transverse aortic constriction (TAC) to observe the contribution of marked adult stem cells to regeneration and remodelling of hypertrophied hearts. Recent studies shows that DOX can suppresses doxorubicin-induced oxidative stress and cellular apoptosis in the murine heart (Lai et al., 2010), which is another cardiovascular disease model commonly used in the field of cardiovascular medicine.

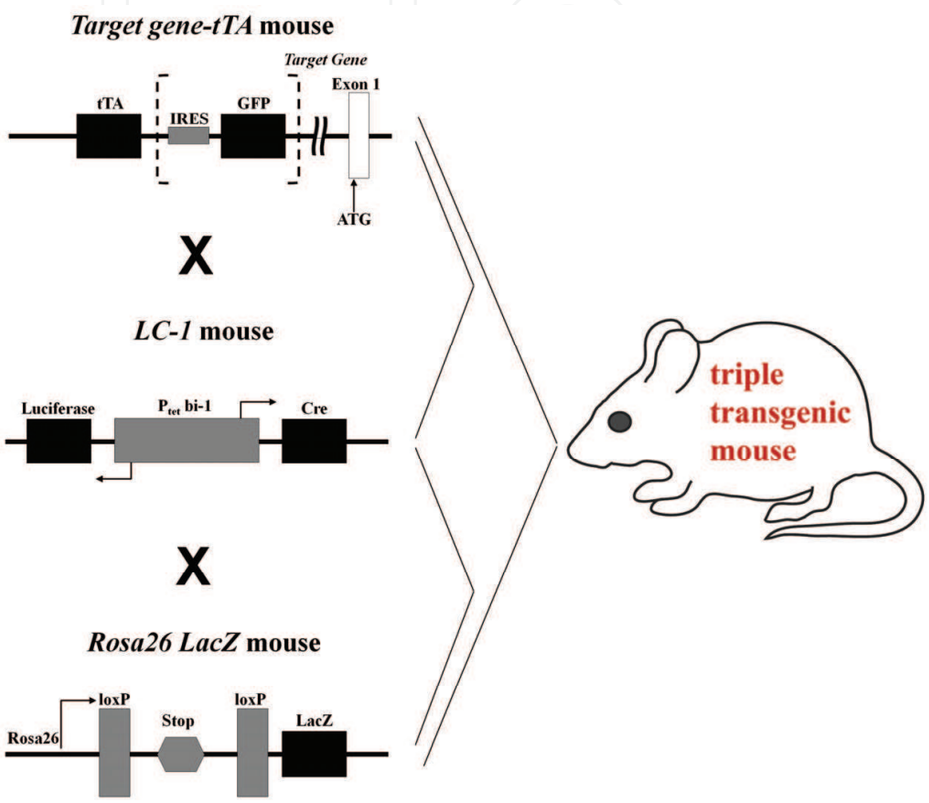


Fig. 1. Triple transgenic mouse model.

To monitor the current expression of a stem cell marker gene, one might insert an additional reporter gene (e.g. GFP) into the genomic locus using an internal ribosomal entry site (IRES) element (Attal et al., 1999). Yet, fluorescent reporter gene will limit the number of fluorescent signals that can be used for further studies to identify the fates of adult stem cells. Furthermore, autofluorescence of the heart caused by lipofuscin, which is breakdown product of old red blood cells (Van de Lest et al., 1995) might obstruct detection of GFP signals, which might lead to misinterpretation of experimental results (Laflamme & Murry, 2005). Therefore, the usage of such reporter genes must be considered carefully.

When such a tTA mouse is created, it should be crossed with a mouse containing a tTA/rtTA responsive element (TRE), such as "Ptet-1" (Baron & Bujard, 2000). In the example shown in Fig. 1, a mouse line called “LC-1”, which ubiquitously expresses Cre-recombinase and luciferase gene in all tissues in an adult mouse upon induction, is shown (Schönig et al., 2002). As shown by Schönig et al. (Schönig et al., 2002), this mouse line was used in the triple transgenic mouse system based on tet-Cre crossed to a reporter line as we propose here. Alternatively, Tet-O-Cre transgenic mice, which expresses Cre-recombinase in a TRE-

dependent manner, might be used (Hsu et al., 2010; Le et al., 2008; Radomska et al., 2002; Tang et al., 2008). The offspring of this mating are called "tet-Cre mice".

Upon successful creation of tet-Cre mice, these mice should be crossed to a reporter line allowing permanent labelling of cells, which have express tTA. Labelling has to be permanent (i.e. based on genomic recombination) to identify derivatives of adult stem cells even after differentiation into mature cells (e.g. cardiomyocyte). Various reporter lines are available to achieve this goal. For example, the Rosa26-LacZ mouse (Soriano, 1999), which expresses β-galactosidase from the Rosa26 locus; Z/AP mice (Lobe et al., 1999), which express β-galactosidase before and human placental alkaline phosphatase (AP) after Cre-mediated recombination. Another option is the Z/EG mouse (Novak et al., 2000), which is similar to the Z/AP mouse, but expresses enhanced GFP instead of AP after Cre-recombinase mediated recombination. Progeny of labelled cells can be easily identified using various cell type specific markers in combination with the reporter system that has been chosen.

Recent studies indicate that transgenes used to label distinct cell types are subject to gene silencing probably due to methylation, which will reduce the efficiency of Cre-mediated labelling (Long & Rossi, 2009). Therefore, it seems prudent to employ at least two different reporter lines. To allow clonal analysis of stem cell derivatives, future approaches will take advantage of reporter mice based on the "*Brainbow*" system. Up to 166 different colours can be generated in these mice by random, alternative use of variant loxP-sites creating combinations of different fluorescent proteins (OFP, M-RFP, M-YFP and M-CFP) (Livet et al., 2007). The original *Brainbow* mice are based on the Thy1 promoter, which restricted its usage to a certain tissues (primarily neuronal cells). Recently, Hans Clevers's group modified the *Brainbow* system by integrating the *Brainbow* cassette into the Rosa26 locus. The resulting "R26R-*Confetti*" mice allow ubiquitous expression in numerous cell types (Snippert et al., 2010). R26R-*Confetti* mice allow random labelling of single adult stem cell with a unique colour. When labelled cells proliferate, the label will be inherited to daughter cells, and it will be possible to distinguish the progeny from that of other individual stem cells. When this strategy is employed to the heart, it should be possible to characterize the identity of adult stem cells, which gives rise to all three lineages of heart (namely, cardiomyocytes, endothelial cells and smooth muscle cells (Moretti et al., 2006)). Further profiling of such cells might facilitate identification of additional markers and effectors.

The tracing system might be combined with different pathological conditions to explore effects of pathogenic stimuli on stem cells. For this purpose, we recently introduce a model to induce right ventricular hypertrophy through pulmonary artery clipping (PAC), which avoids detrimental right ventricular pressure overload, and thus allows long-term survival of operated mice (Kreymborg et al., 2010). Given that the origins of right ventricle and outflow tract are from the secondary heart field (Waldo et al, 2001; Verzi et al., 2005), our PAC model should primarily affect the regions of the secondary heart field so that the behaviour of descendents of Isl1-positive CSCs (Cai et al., 2003; Laugwitz et al., 2005) can be monitored. It is also possible to combine the tracing system with conditional knockout mice. Cre-mediated recombination in such quadruple transgenic mice will allow conditional inactivation of the gene of interest precisely in stem cell-derived, labelled cells.

3.2 Adverse effects of stem cell therapy

Stimulation of cardiac regeneration is one of the major goals for cardiovascular stem cell research. The use of ES cells poises several safety concerns spurred by potential oncogenicity and immunogenicity. Moreover, the generation of ES cells from human

preimplantation embryos has raised several ethical issues. The main problem with other cell types (e.g. BMSCs, skeletal myoblasts) is their limited ability to acquire a fully functional cardiomyocyte state (Haider et al, 2010). Beneficial effects from such cells might be limited to the secretion of cytokines, growth factors and other signaling molecules to provide paracrine and/or trophic effects. Both clinical and basic science studies have provided evidences that some types of cells are able to cause cardiac arrhythmias in patients, although other studies revealed no harmful side-effects . In order to avoid mistakes that tainted gene therapy attempts, it is utmost importance to obtain an optimal control on all types of injected cells. In principal, it seems much safer (and more efficient) to manipulate resident CSCs to differentiate cell types in needs. Such activation of CSCs has been attempted in various studies through injection of growth factors (e.g. Hepatocyte Growth Factor and Insulin-Like Growth Factor-1 (Bocchi et al., 2011) avoiding potential side effects such as arrhythmias). However, it is clear that stimulated signalling pathways needs to be shut off once CSCs have differentiated. The generation of mouse reporter strains to monitor in vivo activation of CSCs would be very helpful. The tracing system that we proposed in the previous subsection should help in this respect

4. Conclusion

Recent findings have challenged the classical view of the heart as a post-mitotic organ. While replacement of cardiomyocytes and other specialized cells of the heart might be taken as granted, much more needs to be learned about the origin of cells that are instrumental for this process. A thorough analysis of resident CSCs in the heart but also of mature cells to contribute to the tissue rejuvenation is of outmost interest. Careful lineage tracing experiments are instrumental to achieve this goal. A potential approach for such an analysis was described in this chapter, which might yield definitive answers about the contribution of different CSCs. Lastly, we would like to answer the question that we asked in the title of this chapter: “Are we there yet?” Our answer is: “No, not yet. But soon, we might be closer.”

5. Acknowledgment

We thank the members of our laboratory (Katharina Jenniches, Pascal Gellert, Mizue Teranishi and David John) for their help and support. We also thank Dr. Petra Uchida and Dr. Stefan Momma for their valuable advice and comments on this book chapter. This work was supported by a start-up-grant of the Excellence Cluster Cardio-Pulmonary System (ECCPS) (to SU), by fellowships of the International Max Planck Research School for Heart and Lung Research (IMPRS-HLR) (to PD-G), and by the Max-Planck-Society, the DFG (Br1416), the EU Commisson (MYORES network of excellence), the Kerckhoff-Foundation and the Excellence Initiative ”Cardiopulmonary System” (to TB).

6. References

Adler, C.P. & Friedburg, H. (1986). Myocardial DNA content, ploidy level and cell number in geriatric hearts: post-mortem examinations of human myocardium in old age. *Journal of Molecular and Cellular Cardiology*, Vol.18, No.1, (January 1986), pp. 39-53.

Aitman, T.J., Critser, J.K., Cuppen, E., Dominiczak, A., Fernandez-Suarez, X.M., Flint, J., Gauguier, D., Geurts, A.M., Gould, M., Harris, P.C., Holmdahl, R., Hubner, N.,

Izsvák, Z., Jacob, H.J., Kuramoto, T., Kwitek, A.E., Marrone, A., Mashimo, T., Moreno, C., Mullins, J., Mullins, L., Olsson, T., Pravenec, M., Riley, L., Saar, K., Serikawa, T., Shull, J.D., Szpirer, C., Twigger, S.N., Voigt, B. & Worley, K. (2008). Progress and prospects in rat genetics: a community view. *Nature Genetics*, Vol.40, No.5 (May 2008), pp. 516-22.

Alaiti, M.A., Ishikawa, M. & Costa, M.A. (2010). Bone marrow and circulating stem/progenitor cells for regenerative cardiovascular therapy. *Translational Research*, Vol.156, No.3 (September 2010), pp. 112-29.

Andersen, D.C., Andersen, P., Schneider, M., Jensen, H.B. & Sheikh, S.P. (2009). Murine "cardiospheres" are not a source of stem cells with cardiomyogenic potential. *Stem Cells*, Vol.27, No.7 (July 2009), pp. 1571-81.

Anversa, P., Kajstura, J., Leri, A. & Bolli, R. (2006). Life and death of cardiac stem cells: a paradigm shift in cardiac biology. *Circulation*, Vol. 113, No.11 (March 2006), pp. 1451-63.

Anversa, P., Leri, A., Rota, M., Hosoda, T., Bearzi, C., Urbanek, K., Kajstura, J. & Bolli, R. (2007). Concise review: stem cells, myocardial regeneration, and methodological artifacts. *Stem Cells*, Vol.25, No.3 (March 2007), pp. 589-601.

Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G. & Isner, J.M. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science*, Vol.275, No.5302 (February 1997), pp. 964-7

Attal, J., Theron, M.C., Puissant, C. & Houdebine, L.M. (1999). Effect of intercistronic length on internal ribosome entry site (IRES) efficiency in bicistronic mRNA. *Gene Expression*, Vol.8, No.5-6, pp. 299-309.

Banerjee, I., Fuseler, J.W., Price, R.L., Borg, T.K. & Baudino, T.A. (2007). Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse. *American Journal of Physiology - Heart and Circulatory Physiology*, Vol.293, No.3 (September 2007), pp. H1883-91.

Barbuti, A., Galvez, B.G., Crespi, A., Scavone, A., Baruscotti, M., Brioschi, C., Cossu, G. & DiFrancesco, D. (2010). Mesoangioblasts from ventricular vessels can differentiate in vitro into cardiac myocytes with sinoatrial-like properties. *Journal of Molecular and Cellular Cardiology*, Vol.48, No.2 (February 2010), pp. 415-23.

Baron, U. & Bujard, H. (2000). Tet repressor based systems for regulated gene expression in eukaryotic cells: principles and advances. *Methods in Enzymology*, Vol.327, pp. 401-421.

Bearzi, C., Rota, M., Hosoda, T., Tillmanns, J., Nascimbene, A., De Angelis, A., Yasuzawa-Amano, S., Trofimova, I., Siggins, R.W., Lecapitaine, N., Cascapera, S., Beltrami, A.P., D'Alessandro, D.A., Zias, E., Quaini, F., Urbanek, K., Michler, R.E., Bolli, R., Kajstura, J., Leri, A. & Anversa, P. (2007). Human cardiac stem cells. *Proceedings of the National Academy of Sciences*, Vol.104, No.35 (August 2007), pp. 14068-73.

Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., Leri, A., Kajstura, J., Nadal-Ginard, B. & Anversa, P. (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*, Vol.114, No.6 (September 2003), pp. 763-76.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H., Jovinge, S. & Frisén, J. (2009).

Evidence for cardiomyocyte renewal in humans. *Science*, Vol.324, No.5923 (April 2009), pp. 98-102.

Bergmann, O., Zdunek, S., Alkass, K., Druid, H., Bernard, S. & Frisén, J. (2011). Identification of cardiomyocyte nuclei and assessment of ploidy for the analysis of cell turnover. *Experimenal Cell Research*, Vol.317, No.2 (January 2011), pp. 188-94.

Bocchi, L., Savi, M., Graiani, G., Rossi, S., Agnetti, A., Stillitano, F., Lagrasta, C., Baruffi, S., Berni, R., Frati, C., Vassalle, M., Squarcia, U., Cerbai, E., Macchi, E., Stilli, D., Quaini, F., & Musso, E. (2011) Growth factor-induced mobilization of cardiac progenitor cells reduces the risk of arrhythmias, in a rat model of chronic myocardial infarction. *PLoS One*, Vol.6, No.3(March 2011), pp. e17750.

Bollini, S., Smart, N. & Riley, P.R. (2011). Resident cardiac progenitor cells: At the heart of regeneration. *Journal of Molecular and Cellular Cardiology*, Vol.50, No.2 (February 2011), pp. 296-303.

Bryder, D., Rossim, D.J. & Weissman, I.L. (2006). Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *American Journal of Pathology*, Vol.169, No.2 (August 2006), pp. 338-46.

Buehr, M., Meek, S., Blair, K., Yang, J., Ure, J., Silva, J., McLay, R., Hall, J., Ying, Q.L. & Smith,

A. (2008). Capture of authentic embryonic stem cells from rat blastocysts. *Cell*, Vol.135, No.7 (December 2008), pp. 1287-98.

Cai, C.L., Liang, X., Shi, Y., Chu, P.H., Pfaff, S.L., Chen, J. & Evans, S. (2003). Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Developmental Cell*, Vol.5, No.6 (December 2003), pp. 877-89.

Carvalho, A.B. & de Carvalho, A.C. (2010). Heart regeneration: Past, present and future. *World Journal of Cardiology*, Vol.2, No.5 (May 2010), pp. 107-11.

Cheng, K., Li, T.S., Malliaras, K., Davis, D.R., Zhang, Y. & Marbán, E. (2010). Magnetic targeting enhances engraftment and functional benefit of iron-labeled cardiosphere-derived cells in myocardial infarction. *Circulation Research*, Vol.106, No.10 (May 2010), pp. 1570-81.

Chugh, A.R,. Zuba-Surma, E.K. & Dawn, B. (2009). Bone marrow-derived mesenchymal stems cells and cardiac repair. *Minerva Cardioangiologica*, Vol.57, No.2 (April 2009), pp. 185-202.

Cox, C.A., Amaral, J., Salloum, R., Guedez, L., Reid, T.W., Jaworski, C., John-Aryankalayil, M., Freedman, K.A., Campos, M.M., Martinez, A., Becerra, S.P. & Carper, D.A. (2010). Doxycycline's effect on ocular angiogenesis: an in vivo analysis. *Ophthalmology*, Vol.117, No.9 (September 2010), pp. 1782-91.

Cui, X., Ji, D., Fisher, D.A., Wu, Y., Briner, D.M. & Weinstein, E.J. (2011). Targeted integration in rat and mouse embryos with zinc-finger nucleases. *Nature Biotechnology*, Vol.29, No.1 (January 2011), pp. 64-7.

Davis, D.R., Kizana, E., Terrovitis, J., Barth, A.S., Zhang, Y., Smith, R.R., Miake, J. & Marbán, E. (2010). Isolation and expansion of functionally-competent cardiac progenitor cells directly from heart biopsies. *Journal of Molecular and Cellular Cardiology*, Vol.49, No.2 (August 2010), pp. 312-21.

Dawn, B., Stein, A.B., Urbanek, K., Rota, M., Whang, B., Rastaldo, R., Torella, D., Tang, X.L., Rezazadeh, A., Kajstura, J., Leri, A., Hunt, G., Varma, J., Prabhu, S.D., Anversa, P.

& Bolli, R. (2005). Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proceedings of the National Academy of Sciences*, Vol.102, No.10 (March 2005), pp. 3766-71.

Devbhandari, M.P., Meraj, S., Jones, M.T., Kadir, I. & Bridgewater, B. (2007). Primary cardiac sarcoma: reports of two cases and a review of current literature. *Journal of Cardiothoracic Surgery*, Vol.2 (July 2007), pp. 34.

Di Nardo, P., Forte, G., Ahluwalia, A. & Minieri, M. (2010). Cardiac progenitor cells: potency and control. *Journal of Cellular Physiology*, Vol.224, No.3 (September 2010), pp. 590-600.

DuFrain, R.J., McFee, A.F., Linkous, S., Jennings, C.J. & Lowe, K.W. (1984). In vivo SCE analysis using bromodeoxyuridine, iododeoxyuridine, and chlorodeoxyuridine. *Mutation Research*, Vol.139, No.2 (February 1984), pp. 57-60.

Ebelt, H., Hufnagel, N., Neuhaus, P., Neuhaus, H., Gajawada, P., Simm, A., Müller-Werdan, U., Werdan, K. & Braun, T. (2005). Divergent siblings: E2F2 and E2F4 but not E2F1 and E2F3 induce DNA synthesis in cardiomyocytes without activation of apoptosis. *Circulation Research*, Vol.96, No.5 (March 2005), pp. 509-17.

Ebelt, H., Liu, Z., Müller-Werdan, U., Werdan, K. & Braun, T. (2006). Making omelets without breaking eggs: E2F-mediated induction of cardiomyoycte cell proliferation without stimulation of apoptosis. *Cell Cycle*, Vol.5, No.21 (November 2006), pp. 2436-9.

Ebelt, H., Zhang, Y., Köhler, K., Xu, J., Gajawada, P., Boettger, T., Hollemann, T., Müller-Werdan, U., Werdan, K. & Braun, T. (2008). Directed expression of dominant-negative p73 enables proliferation of cardiomyocytes in mice. *Journal of Molecular and Cellular Cardiology*, Vol.45, No.3 (September 2008), pp. 411-9.

Ebelt, H., Zhang, Y., Kampke, A., Xu, J., Schlitt, A., Buerke, M., Müller-Werdan, U., Werdan, K. & Braun, T. (2008). E2F2 expression induces proliferation of terminally differentiated cardiomyocytes in vivo. Cardiovascular Research, Vol.80, No.2 (November 2008), pp. 219-26.

Ellison, G.M., Galuppo, V., Vicinanza, C., Aquila, I., Waring, C.D., Leone, A., Indolfi, C. & Torella, D. (2010). Cardiac stem and progenitor cell identification: different markers for the same cell? *Frontier in Bioscience (Scholor Edition)*, Vol.2 (January 2010), pp. 641-52.

Ergün, S., Tilki, D. & Klein, D. (2011). Vascular Wall as a Reservoir for Different Types of Stem and Progenitor Cells. *Antioxidants & Redox Signaling*, [Epub ahead of print] (January 2011).

Errami, M., Galindo, C.L., Tassa, A.T., Dimaio, J.M., Hill, J.A. & Garner, H.R. (2008). Doxycycline attenuates isoproterenol- and transverse aortic banding-induced cardiac hypertrophy in mice. *Journal of Pharmacology and Experimental Therapeutics*, Vol.324, No.3 (March 2008), pp. 1196-203.

Eyden, B. (2004). Fibroblast phenotype plasticity: relevance for understanding heterogeneity in "fibroblastic" tumors. *Ultrastructural Pathology*, Vol.28, No.5-6 (September-December 2004), pp. 307-19.

Fainaru, O., Adini, I., Benny, O., Bazinet, L., Pravda, E., D'Amato, R. & Folkman, J. (2008). Doxycycline induces membrane expression of VE-cadherin on endothelial cells and

prevents vascular hyperpermeability. *FASEB Journal*, Vol.22, No.10 (October 2008), pp. 3728-35.

Forte, G., Carotenuto, F., Pagliari, F., Pagliari, S., Cossa, P., Fiaccavento, R., Ahluwalia, A., Vozzi, G., Vinci, B., Serafino, A., Rinaldi, A., Traversa, E., Carosella, L., Minieri, M. & Di Nardo, P. (2008). Criticality of the biological and physical stimuli array inducing resident cardiac stem cell determination. *Stem Cells*, Vol.26, No.8 (August 2008), pp. 2093-103.

Friedenstein, A.J., Gorskaja, J.F. & Kulagina, N.N. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Experimental Hematology*, Vol.4, No.5 (September 1976), pp. 267-74.

Galvez, B.G., Sampaolesi, M., Barbuti, A., Crespi, A., Covarello, D., Brunelli, S., Dellavalle, A., Crippa, S., Balconi, G., Cuccovillo, I., Molla, F., Staszewsky, L., Latini, R., Difrancesco, D. & Cossu, G. (2008). Cardiac mesoangioblasts are committed, self-renewable progenitors, associated with small vessels of juvenile mouse ventricle. *Cell Death and Differetiation*, Vol.15, No.9 (September 2008), pp. 1417-28.

Gálvez, B.G., Covarello, D., Tolorenzi, R., Brunelli, S., Dellavalle, A., Crippa, S., Mohammed, S.A., Scialla, L., Cuccovillo, I., Molla, F., Staszewsky, L., Maisano, F., Sampaolesi, M., Latini, R. & Cossu, G. (2009). Human cardiac mesoangioblasts isolated from hypertrophic cardiomyopathies are greatly reduced in proliferation and differentiation potency. *Cardiovascular Research*, Vol.83, No.4 (September 2009), pp. 707-16.

Geurts, A.M., Cost, G.J., Freyvert, Y., Zeitler, B., Miller, J.C., Choi, V.M., Jenkins, S.S., Wood, A., Cui, X., Meng, X., Vincent, A., Lam, S., Michalkiewicz, M., Schilling, R., Foeckler, J., Kalloway, S., Weiler, H., Ménoret, S., Anegon, I., Davis, G.D., Zhang, L., Rebar, E.J., Gregory, P.D., Urnov, F.D., Jacob, H.J. & Buelow, R. (2009). Knockout rats via embryo microinjection of zinc-finger nucleases. *Science*, Vol.325, No.5939 (July 2009), pp. 433

Gossen, M. & Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proceedings of the National Academy of Sciences*, Vol.89, No.12 (June 1992), pp. 5547-51.

Haider, K.H., Buccini, S., Ahmed, R.P., & Ashraf, M. (2010) De novo myocardial regeneration: advances and pitfalls. *Antioxidants & Redox Signaling*, Vol.13, No.12 (December 2010), pp. 1867-77.

Holaska, J.M. (2008). Emerin and the nuclear lamina in muscle and cardiac disease. *Circulation Research*, Vol.103, No.1 (July 2008), pp. 16-23.

Hsieh, P.C., Segers, V.F., Davis, M.E., MacGillivray, C., Gannon, J., Molkentin, J.D., Robbins, J. & Lee, R.T. (2007). Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nature Medicine*, Vol.13, No.8 (August 2007), pp. 970-4.

Hsu, W., Mirando, A.J. & Yu, H.M. (2010). Manipulating gene activity in Wnt1-expressing precursors of neural epithelial and neural crest cells. *Developmental Dynamics*, Vol.239, No.1 (January 2010), pp. 338-45.

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., Rota, M., Del Monte, F., Orlic, D., Tisdale, J., Leri, A. & Anversa, P. (2010). Cardiomyogenesis in the adult human heart. *Circulation Research*, Vol.107, No.2 (July 2010), pp. 305-15.

Kajstura, J., Gurusamy, N., Ogórek, B., Goichberg, P., Clavo-Rondon, C., Hosoda, T., D'Amario, D., Bardelli, S., Beltrami, A.P., Cesselli, D., Bussani, R., Del Monte, F., Quaini, F., Rota, M., Beltrami, C.A., Buchholz, B.A., Leri, A. & Anversa, P. (2010). Myocyte Turnover in the Aging Human Heart. *Circulation Research*, Vol.107, No.11 (November 2010), pp. 1374-86.

Karsner, H.T., Saphir, O. & Todd, T.W. (1925). The State of the Cardiac Muscle in Hypertrophy and Atrophy. *American Journal of Pathology*, Vol.1, No.4 (July 1925), pp. 351-372.1.

Kreymborg, K., Uchida, S., Gellert, P., Schneider, A., Boettger, T., Voswinckel, R., Wietelmann, A., Szibor, M., Weissmann, N., Ghofrani, A.H., Schermuly, R., Schranz, D., Seeger, W. & Braun, T. (2010). Identification of right heart-enriched genes in a murine model of chronic outflow tract obstruction. *Journal of Molecular and Cellular Cardiology*, Vol.49, No.4 (October 2010), pp. 598-605.

Kullo, I.J. & Cooper, L.T. (2010). Early identification of cardiovascular risk using genomics and proteomics. *Nature Review Cardiology*, Vol.7, No.6 (June 2010), pp. 309-17.

Kumar, A.H. & Caplice, N.M. (2010). Clinical potential of adult vascular progenitor cells. *Arteriosclerosis, Thrombosis, and Vascular Biology*, Vol.30, No.6 (June 2010), pp. 1080-7.

Laflamme, M.A. & Murry, C.E. (2005). Regenerating the heart. *Nature Biotechnology*, Vol.23, No.7 (July 2005), pp. 845-56.

Lai, H.C., Yeh, Y.C., Ting, C.T., Lee, W.L., Lee, H.W., Wang, L.C., Wang, K.Y., Lai, H.C., Wu, A. & Liu, T.J. (2010). Doxycycline suppresses doxorubicin-induced oxidative stress and cellular apoptosis in mouse hearts. *European Journal of Pharmacology*, Vol.644, No.1-3(October 2010), pp. 176-87.

Laugwitz, K.L., Moretti, A., Lam, J., Gruber, P., Chen, Y., Woodard, S., Lin, L.Z., Cai, C.L., Lu, M.M., Reth, M., Platoshyn, O., Yuan, J.X., Evans, S. & Chien, K.R. (2005). Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature*, Vol.433, No.7026 (February 2005), pp. 647-53.

Le, Y.Z., Zheng, W., Rao, P.C., Zheng, L., Anderson, R.E., Esumi, N., Zack, D.J. & Zhu, M. (2008). Inducible expression of cre recombinase in the retinal pigmented epithelium. *Investigative Ophthalmology & Visual Science*, Vol.49, No.3 (March 2008), pp. 1248-53.

Li, P., Tong, C., Mehrian-Shai, R., Jia, L., Wu, N., Yan, Y., Maxson, R.E., Schulze, E.N., Song, H., Hsieh, C.L., Pera, M.F. & Ying, Q.L. (2008). Germline competent embryonic stem cells derived from rat blastocysts. *Cell*, Vol.135, No.7 (December 2008), pp. 1299-310.

Liang, S.X., Tan, T.Y., Gaudry, L. & Chong, B. (2010). Differentiation and migration of Sca1+/CD31- cardiac side population cells in a murine myocardial ischemic model. *International Journal of Cardiology*, Vol.138, No.1 (January 2010), pp. 40-9.

Limana, F., Capogrossi, M.C. & Germani, A. (2011). The epicardium in cardiac repair: from the stem cell view. *Pharmacology & Therapeutics*, Vol.129, No.1 (January 2011), pp. 82-96.

Linke, A., Müller, P., Nurzynska, D., Casarsa, C., Torella, D., Nascimbene, A., Castaldo, C., Cascapera, S., Böhm, M., Quaini, F., Urbanek, K., Leri, A., Hintze, T.H., Kajstura, J. & Anversa, P. (2005). Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function.

Proceedings of the National Academy of Sciences, Vol.102, No.25 (June 2005), pp. 8966-71.

Liu, Z., Yue, S., Chen, X., Kubin, T. & Braun, T. (2010). Regulation of cardiomyocyte polyploidy and multinucleation by CyclinG1. *Circulation Research*, Vol.106, No.9 (May 2010), pp. 1498-506.

Livet, J., Weissman, T.A., Kang, H., Draft, R.W., Lu, J., Bennis, R.A., Sanes, J.R. & Lichtman, J.W. (2007). Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature*, Vol.450, No.7166 (November 2007), pp. 56-62.

Lobe, C.G., Koop, K.E., Kreppner, W., Lomeli, H., Gertsenstein, M. & Nagy, A. (1999). Z/AP, a double reporter for cre-mediated recombination. *Developmental Biology*, Vol.208, No.2 (April 1999), pp. 281-92.

Long, M.A. & Rossi, F.M. (2009). Silencing inhibits Cre-mediated recombination of the Z/AP and Z/EG reporters in adult cells. *PLoS One*, Vol.4, No.5 (May 2009), pp. e5435.

Martin, C.M., Meeson, A.P., Robertson, S.M., Hawke, T.J., Richardson, J.A., Bates, S., Goetsch, S.C., Gallardo, T.D. & Garry, D.J. (2004). Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Developmental Biology*, Vol.265, No.1 (January 2004), pp. 262-75.

Matsuura, K., Nagai, T., Nishigaki, N., Oyama, T., Nishi, J., Wada, H., Sano, M., Toko, H., Akazawa, H., Sato, T., Nakaya, H., Kasanuki, H. & Komuro, I. (2004). Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *Journal of Biological Chemistry*, Vol.279, No.12 (March 2004), pp. 11384-91.

Messina, E., De Angelis, L., Frati, G., Morrone, S., Chimenti, S., Fiordaliso, F., Salio, M., Battaglia, M., Latronico, M.V., Coletta, M., Vivarelli, E., Frati, L., Cossu, G. & Giacomello, A. (2004). Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circulation Research*, Vol.95, No.9 (October 2004), pp. 911-21.

Miyamoto, S., Kawaguchi, N., Ellison, G.M., Matsuoka, R., Shin'oka, T. & Kurosawa, H. (2010). Characterization of long-term cultured c-kit+ cardiac stem cells derived from adult rat hearts. *Stem Cells and Development*, Vol.19, No.1 (January 2010), pp. 105-16.

Morabito, C.J., Dettman, R.W., Kattan, J., Collier, J.M. & Bristow, J. (2001). Positive and negative regulation of epicardial-mesenchymal transformation during avian heart development. *Developmental Biology*, Vol.234, No.1 (June 2001), pp. 204-15.

Moretti, A., Caron, L., Nakano, A., Lam, J.T., Bernshausen, A., Chen, Y., Qyang, Y., Bu, L., Sasaki, M., Martin-Puig, S., Sun, Y., Evans, S.M., Laugwitz, K.L. & Chien, K.R. (2006). Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell*, Vol.127, No.6 (December 2006), pp. 1151-65.

Musunuru, K., Domian, I.J. & Chien, K.R. (2010). Stem cell models of cardiac development and disease. *Annual Review of Cell and Developmental Biology*, Vol.26 (November 2010), pp. 667-87.

Nag, A.C. (1980). Study of non-muscle cells of the adult mammalian heart: a fine structural analysis and distribution. *Cytobios*, Vol.28, No.109, pp. 41-61.

Nern, C., Wolff, I., Macas, J., von Randow, J., Scharenberg, C., Priller, J. & Momma, S. (2009). Fusion of hematopoietic cells with Purkinje neurons does not lead to stable

heterokaryon formation under noninvasive conditions. *Journal of Neuroscience*, Vol.29, No.12 (March 2009), pp. 3799-807.

Novak, A., Guo, C., Yang, W., Nagy, A. & Lobe, C.G. (2000). Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis*, Vol.28, No.3-4 (November-December 2000), pp. 147-55.

Oh, H., Bradfute, S.B., Gallardo, T.D., Nakamura, T., Gaussin, V., Mishina, Y., Pocius, J., Michael, L.H., Behringer, R.R., Garry, D.J., Entman, M.L. & Schneider, M.D. (2003). Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proceedings of the National Academy of Sciences*, Vol.100, No.21 (October 2003), pp. 12313-8.

Olivetti, G., Giordano, G., Corradi, D., Melissari, M., Lagrasta, C., Gambert, S.R. & Anversa, P. (1995). Gender differences and aging: effects on the human heart. *Journal of the American College of Cardiology*, Vol.26, No.4 (October 1995), pp. 1068-79.

Olivetti, G., Cigola, E., Maestri, R., Corradi, D., Lagrasta, C., Gambert, S.R. & Anversa, P. (1996). Aging, cardiac hypertrophy and ischemic cardiomyopathy do not affect the proportion of mononucleated and multinucleated myocytes in the human heart. *Journal of Molecular and Cellular Cardiology*, Vol.28, No.7 (July 1996), pp. 1463-77.

Owens, G.K., Kumar, M.S. & Wamhoff, B.R. (2004). Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiological Reviews*, Vol.84, No.3 (July 2004), pp. 767-801.

Owens, G.K. (1995). Regulation of differentiation of vascular smooth muscle cells. *Physiological Reviews*, Vol.75, No.3 (July 1995), pp. 487-517.

Oyama, T., Nagai, T., Wada, H., Naito, A.T., Matsuura, K., Iwanaga, K., Takahashi, T., Goto, M., Mikami, Y., Yasuda, N., Akazawa, H., Uezumi, A., Takeda, S. & Komuro, I. (2007). Cardiac side population cells have a potential to migrate and differentiate into cardiomyocytes in vitro and in vivo. *Journal of Cell Biology*, Vol.176, No.3 (January 2007), pp. 329-41.

Parmacek, M.S. & Epstein, J.A. (2009). Cardiomyocyte renewal. *New England Journal of Medicine*, Vol.361, No.1 (July 2009), pp. 86-8.

Petrich, B.G., Molkentin, J.D. & Wang, Y. (2003). Temporal activation of c-Jun N-terminal kinase in adult transgenic heart via cre-loxP-mediated DNA recombination. *FASEB Journal*, Vol.17, No.6 (April 2003), pp. 749-51.

Pfister, O., Mouquet, F., Jain, M., Summer, R., Helmes, M., Fine, A., Colucci, W.S. & Liao, R. (2005). CD31- but Not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circulation Research*, Vol.97, No.1 (July 2007), pp. 52-61.

Phinney, D.G. & Prockop, D.J. (2007). Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views. *Stem Cells*, Vol.25, No.11 (November 2007), pp. 2896-902.

Prokopi, M., Pula, G., Mayr, U., Devue, C., Gallagher, J., Xiao, Q., Boulanger, C.M., Westwood, N., Urbich, C., Willeit, J., Steiner, M., Breuss, J., Xu, Q., Kiechl, S. & Mayr, M. (2009). Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. *Blood*, Vol.114, No.3 (July 2009), pp. 723-32.

Psaltis, P.J., Zannettino, A.C., Worthley, S.G. & Gronthos, S. (2008). Concise review: mesenchymal stromal cells: potential for cardiovascular repair. *Stem Cells*, Vol.26, No.9 (September 2008), pp. 2201-10.

Psaltis, P.J., Harbuzariu, A., Delacroix, S., Holroyd, E.W. & Simari, R.D. (2010). Resident Vascular Progenitor Cells-Diverse Origins, Phenotype, and Function. *Journal of Cardiovascular Translational Research*, Vol.4, No.2 (April 2011), pp. 161-76.

Radomska, H.S., Gonzalez, D.A., Okuno, Y., Iwasaki, H., Nagy, A., Akashi, K., Tenen, D.G. & Huettner, C.S. (2002). Transgenic targeting with regulatory elements of the human CD34 gene. *Blood*, Vol.100, No.13 (December 2002), pp. 4410-9.

Ratajczak, M.Z., Kucia, M., Ratajczak, J. & Zuba-Surma, E.K. (2009). A multi-instrumental approach to identify and purify very small embryonic like stem cells (VSELs) from adult tissues. *Micron*, Vol.40, No.3 (April 2009), pp. 386-93.

Richardson, M.R. & Yoder, M.C. (2011). Endothelial progenitor cells: Quo Vadis? *Journal of Molecular and Cellular Cardiology*, Vol.50, No.2 (February 2011), pp. 266-72.

Rios, M. & Williams, D.A. (1990). Systematic analysis of the ability of stromal cell lines derived from different murine adult tissues to support maintenance of hematopoietic stem cells in vitro. *Journal of Cellular Physiology*, Vol.145, No.3 (December 1990), pp. 434-43.

Rosenblatt-Velin, N., Lepore, M.G., Cartoni, C., Beermann, F. & Pedrazzini, T. (2005). FGF-2 controls the differentiation of resident cardiac precursors into functional cardiomyocytes. *Journal Clinical Investigation*, Vol.115, No.7 (July 2005), pp. 1724-33.

Rubart, M. & Field, L.J. (2006). Cardiac regeneration: repopulating the heart. *Annual Review of Physiology*, Vol.68, pp. 29-49.

Rzucidlo, E.M., Martin, K.A. & Powell, R.J. (2007). Regulation of vascular smooth muscle cell differentiation. *Journal of Vascular Surgery*, Vol.45, Suppl.A (June 2007), pp. A25-32.

Schönig, K., Schwenk, F., Rajewsky, K. & Bujard, H. (2002). Stringent doxycycline dependent control of CRE recombinase in vivo. *Nucleic Acids Research*, Vol.30, No.23 (December 2002), pp. e134.

Schulze, M., Belema-Bedada, F., Technau, A. & Braun, T. (2005). Mesenchymal stem cells are recruited to striated muscle by NFAT/IL-4-mediated cell fusion. *Genes & Development*, Vol.19, No.15 (August 2005), pp. 1787-98.

Smedley, D., Salimova, E. & Rosenthal, N. (2010). Cre recombinase resources for conditional mouse mutagenesis. *Methods*, Vol.53, No.4 (April 2011), pp. 411-6.

Smith, R.R., Barile, L., Cho, H.C., Leppo, M.K., Hare, J.M., Messina, E., Giacomello, A., Abraham, M.R. & Marbán, E. (2007). Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*, Vol.115, No.7 (February 2007), pp. 896-908.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D. & Clevers, H. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell*, Vol.143, No.1 (October 2010), pp. 134-44.

Soonpaa, M.H. & Field, L.J. (1998). Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circulation Research*, Vol.83, No.1 (July 1998), pp. 15-26.

Soonpaa, M.H., Kim, K.K., Pajak, L., Franklin, M. & Field, L.J. (1996). Cardiomyocyte DNA synthesis and binucleation during murine development. *American Journal of Physiology*, Vol.271, No.5 Pt 2 (November 1996), pp. H2183-9.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nature Genetics*, Vol.21, No.1 (January 1999), pp. 70-1.

Stamm, C., Choi, Y.H., Nasseri, B. & Hetzer, R. (2009). A heart full of stem cells: the spectrum of myocardial progenitor cells in the postnatal heart. *Therapeutic Advances in Cardiovascular Disease*, Vol.3, No.3 (2009), pp. 215-29.

Stary, E., Gaupp, R., Lechner, S., Leibig, M., Tichy, E., Kolb, M. & Bertram, R. (2010). New architectures for Tet-on and Tet-off regulation in Staphylococcus aureus. *Applied and Environmental Microbiology*, Vol.76, No.3 (February 2010), pp. 680-7.

Stintzing, S., Ocker, M., Hartner, A., Amann, K., Barbera, L. & Neureiter, D. (2009). Differentiation patterning of vascular smooth muscle cells (VSMC) in atherosclerosis. *Virchows Archiv*, Vol.455, No.2 (August 2009), pp. 171-85.

Tallini, Y.N., Greene, K.S., Craven, M., Spealman, A., Breitbach, M., Smith, J., Fisher, P.J., Steffey, M., Hesse, M., Doran, R.M., Woods, A., Singh, B., Yen, A., Fleischmann, B.K. & Kotlikoff, M.I. (2009). c-kit expression identifies cardiovascular precursors in the neonatal heart. *Proceedings of the National Academy of Sciences*, Vol.106, No.6 (February 2009), pp. 1808-13.

Tang, W., Zeve, D., Suh, J.M., Bosnakovski, D., Kyba, M., Hammer, R.E., Tallquist, M.D. & Graff, J.M. (2008). White fat progenitor cells reside in the adipose vasculature. *Science*, Vol.322, No.5901 (October 2008), pp. 583-6.

Tateishi, K., Ashihara, E., Takehara, N., Nomura, T., Honsho, S., Nakagami, T., Morikawa, S., Takahashi, T., Ueyama, T., Matsubara, H. & Oh, H. (2007). Clonally amplified cardiac stem cells are regulated by Sca-1 signaling for efficient cardiovascular regeneration. *Journal of Cell Science*, Vol.120, No.Pt 10 (May 2007), pp. 1791-800.

Tateishi, K., Takehara, N., Matsubara, H. & Oh, H. (2008). Stemming heart failure with cardiac- or reprogrammed-stem cells. *Journal of Cellular and Molecular Medicine*, Vol.12, No.6A (December 2008), pp. 2217-32.

Tillmanns, J., Rota, M., Hosoda, T., Misao, Y., Esposito, G., Gonzalez, A., Vitale, S., Parolin, C., Yasuzawa-Amano, S., Muraski, J., De Angelis, A., Lecapitaine, N., Siggins, R.W., Loredo, M., Bearzi, C., Bolli, R., Urbanek, K., Leri, A., Kajstura, J. & Anversa, P. (2008). Formation of large coronary arteries by cardiac progenitor cells. *Proceedings of the National Academy of Sciences*, Vol.105, No.5 (February 2008), pp. 1668-73.

Tong, C., Li, P., Wu, N.L., Yan, Y. & Ying, Q.L. (2010). Production of p53 gene knockout rats by homologous recombination in embryonic stem cells. *Nature*, Vol.467, No.7312 (September 2010), pp. 211-3.

Urbanek, K., Quaini, F., Tasca, G., Torella, D., Castaldo, C., Nadal-Ginard, B., Leri, A., Kajstura, J., Quaini, E. & Anversa, P. (2003). Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proceedings of the National Academy of Sciences*, Vol.100, No.18 (September 2003), pp. 10440-5.

Urlinger, S., Baron, U., Thellmann, M., Hasan, M.T., Bujard, H. & Hillen, W. (2000). Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. *Proceedings of the National Academy of Sciences*, Vol.97, No.14 (July 2000), pp. 7963-8.

Van de Lest, C.H., Versteeg, E.M., Veerkamp, J.H. & Van Kuppevelt, T.H. (1995). Elimination of autofluorescence in immunofluorescence microscopy with digital image processing. *Journal of Histochemistry and Cytochemistry*, Vol.43, No.7 (1995), pp. 727-30.

Verzi, M.P., McCulley, D.J., De Val, S., Dodou, E. & Black, B.L. (2005). The right ventricle, outflow tract, and ventricular septum comprise a restricted expression domain

within the secondary/anterior heart field. *Developmental Biology*, Vol.287, No.1 (November 2005), pp. 134-45.

Waldo, K.L., Kumiski, D.H., Wallis, K.T., Stadt, H.A., Hutson, M.R., Platt, D.H. & Kirby, M.L. (2001). Conotruncal myocardium arises from a secondary heart field. *Development*, Vol.128, No.16 (August 2001), pp. 3179-88.

Wang, X., Hu, Q., Nakamura, Y., Lee, J., Zhang, G., From, A.H. & Zhang, J. (2006) The role of the sca-1+/CD31- cardiac progenitor cell population in postinfarction left ventricular remodeling. *Stem Cells*, Vol.24, No.7 (July 2006), pp. 1779-88.

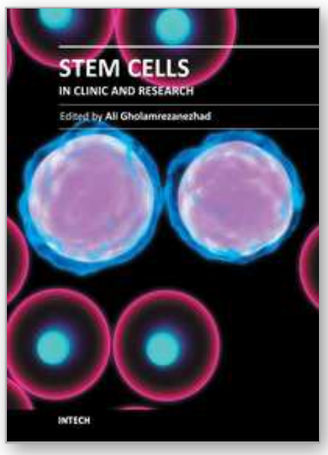
Wen, Z., Zheng, S., Zhou, C., Wang, J. & Wang, T. (2010). Repair mechanisms of bone marrow mesenchymal stem cells in myocardial infarction. *Journal of Cellular and Molecular Medicine*, (December 2010) [Epub ahead of print].

Wessels, A. & Pérez-Pomares, J.M. (2004). The epicardium and epicardially derived cells (EPDCs) as cardiac stem cells. *Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology*, Vol.276, No.1 (January 2004), pp. 43-57.

Wu, S.M., Fujiwara, Y., Cibulsky, S.M., Clapham, D.E., Lien, C.L., Schultheiss, T.M. & Orkin, S.H. (2006). Developmental origin of a bipotential myocardial and smooth muscle cell precursor in the mammalian heart. *Cell*, Vol.127, No.6 (December 2006), pp. 1137-50.

Zeisberg, E.M., Tarnavski, O., Zeisberg, M., Dorfman, A.L., McMullen, J.R., Gustafsson, E., Chandraker, A., Yuan, X., Pu, W.T., Roberts, A.B., Neilson, E.G., Sayegh, M.H., Izumo, S. & Kalluri, R. (2007). Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nature Medicine*, Vol.13, No.8 (August 2007), pp. 952-61.

IntechOpen



Stem Cells in Clinic and Research

Edited by Dr. Ali Gholamrezanezhad

ISBN 978-953-307-797-0
Hard cover, 804 pages
Publisher InTech
Published online 23, August, 2011
Published in print edition August, 2011

Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Shizuka Uchida, Piera De Gaspari and Thomas Braun (2011). Are We There Yet? A Story About Cardiac Stem Cells, Stem Cells in Clinic and Research, Dr. Ali Gholamrezanezhad (Ed.), ISBN: 978-953-307-797-0, InTech, Available from: http://www.intechopen.com/books/stem-cells-in-clinic-and-research/are-we-there-yet-a-story-about-cardiac-stem-cells

INTECH
open science | open minds

www.intechopen.com

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen

IntechOpen

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen