

Cardiac regeneration: different cells same goal

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Abstract Cardiovascular diseases are the leading cause of mortality, morbidity, hospitalization and impaired quality of life. In most, if not all, pathologic cardiac ischemia ensues triggering a succession of events leading to massive death of cardiomyocytes, fibroblast and extracellular matrix accumulation, cardiomyocyte hypertrophy which culminates in heart failure and eventually death. Though current pharmacological treatment is able to delay the succession of events and as a consequence the development of heart failure, the only currently available and effective treatment of end-stage heart failure is heart transplantation. However, donor heart availability and immunorejection upon transplantation seriously limit the applicability. Cardiac regeneration could provide a solution, making real a dream of both scientist and clinician in the previous century and ending an ongoing challenge for this century. In this review, we present a basic overview of the various cell types that have been used in both the clinical and research setting with respect to myocardial differentiation.

Keywords Cardiac regeneration · Embryonic stem cells · Somatic stem cells · Cardiomyocytes · Cardiac development

1 Myocardial regeneration

A little over a decade ago, it was generally accepted that the human heart is a terminally differentiated organ lacking any intrinsic regenerative capacity. After birth, the cardiomyocytes undergo a final round of cell division with a portion of the cardiomyocytes becoming binuclear and/or polyploid [14, 82, 83]. The observed post-natal growth is attributed to mainly to hypertrophic growth of the resident cardiomyocytes [76], with very no contribution of cell division. In line with these findings it was observed that amputation of the ventricular apex at 1 day after birth in mice tolerates full regeneration of this region, whereas at 7 days post natal this capacity is completely lost [57].

The dogma that the adult heart is a post-mitotic organ, was challenged for the first time in 1998 by Kajstura et al. [37], who showed that a small (0.001%) portion of cardiomyocytes undergo mitosis in normal hearts. Though numerous subsequent follow-up studies have reported slightly differing numbers, the issue remained controversial. Bergmann et al. [12], finally added real weight to the idea that cardiomyocytes can and do undergo mitosis in adult hearts, using C14 radiocarbon dating of human genomic DNA. This experiment resembles that of a pulse-chase experiment in which genomic DNA was labelled as a result of trace amounts of radioactive nuclear fallout produced as a result of nuclear weapons testing during the cold war. According to their study, at the age of 25 cardiomyocytes have a mitotic turn-over of approximately 1% per year. Although this value gradually decreases with age, the numbers suggest that about 50% of resident cardiomyocytes are renewed during life. These findings were also found to be in line with mouse data [66]. However, the initial challengers of the dogma, revisited and expanded their initial study using state of the art tools [38] reporting a

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similar decrease in myocyte turn-over rate with age. Interestingly, based on their methods and estimations the cardiomyocytes of the heart would be renewed 10-times during a normal life span.

Although this long lasting discussion has regained a renewed interest, it is evident that the adult human heart has a limited endogenous regenerative capacity. This capacity is insufficient (1) to maintain the number of cardiomyocytes during aging and (2) to replace cardiomyocytes lost upon injury and/or disease, leading to decreased heart function and eventually heart failure. This contrasts sharply to the high regenerative capacity found in teleosts and amphibians [6]. A second question directly relating to this endogenous regenerative capacity is the cellular origin of the newly formed cardiomyocytes. The regenerated cardiomyocytes are either derived from cells located within or outside the heart. The quest for progenitors of cardiomyocytes within the heart has led to the identification of a multitude of cardiac stem cells or progenitors, each with a different molecular signature [41, 53]. cKit (CD117) and Flk-1 are but two of the markers used to identify precursor cells contributing to the cardiomyocyte, smooth muscle and endocardial lineage. FACS sorting of the cKit-positive population revealed that their contribution to the heart gradually decreases with age, from 0.65%, of which 10% is CD45-(hematopoietic)positive, in neonatal mouse heart to 0.5%, of which 15% is CD45-positive, in the adult heart [88]. Such cells can also be isolated from the human heart as well. Though absolute numbers are not known, they are rare and their number also decreases with age [29, 30]. Co-culturing neonatal cKit-positive cells with foetal cardiomyocytes results in robust differentiation into cardiomyocytes, whereas adult cKit-positive cells appear to have lost this capacity. In vivo testing using mouse models revealed that injecting these cells into an infarct showed no significant differentiation into cardiomyocytes [10, 29, 30, 88].

Though it is currently accepted that cardiac stem cells or progenitors exist, it is as yet not known what factors are needed to expand this population and to induce their differentiation into functional cardiomyocytes.

2 (Human) Embryonic stem cells derivation and potential

With the derivation of the first human embryonic stem cells (hESC) by Thomson et al. [73], the field of stem cell research caught the scientific and public domain's attention, bringing an understandable high level of hope for new breakthroughs in regenerative medicine. Despite the ensuing reactions regarding the ethical issues that surround the isolation and use of cells that are of a human embryonic origin, the prospect of being able to treat a multiplicity of

diseases, particularly the appeal of finally being able to restore function of tissue lost during a myocardial infarct, stem cell research took off at an incredible rate (Fig. 1). However, apart from the ethical issues surrounding this research, hESC, as we shall discuss, have thrown up many obstacles on their route to the clinic resulting in a re-evaluation of strategies being taken to implement their use. Although other 'stem cell' sources have been applied more readily in the clinical setting, hESCs have only just received the first green light for an FDA approved human trial to investigate their applicability for the treatment of complete thoracic spinal severance [2].

Embryonic stem cells are derived from the inner cell mass of the blastocyst ~5 days post fertilization. Given the diverse and sometimes incorrect use of the word stem cell, ESC were described using the physical characteristics (i) derived from the pre-implanted embryo, (ii) prolonged, undifferentiated proliferation and (iii) the potential to develop and give rise to all three germinal layers (endoderm, mesoderm and ectoderm) [72]. The molecular definition of hESC remains a challenge, due in part to the heterogeneity that already exists even at the blastocyst stage. This is perhaps not surprising since the inner cell mass will rapidly give rise to the ordered three layered germinal disc from which the embryo will develop. Cells may therefore even at this stage differ from their neighbouring cells and begin to display the molecular characteristics of one of the germinal layers, which in turn could be composed of cells that differ from each other. However, markers, have been identified which serve as tools for sorting and cellular selection.

The two glycosphingolipid surface antigen markers SSEA-3 and SSEA-4 are, are identified on human embryonal carcinoma (EC) cell lines, on early cell-cleavage mouse embryos [40] and later on cells of the inner cell mass of human blastocysts [33]. In contrast to mice, which have lost expression of these antigens by the blastocyst stage, these markers along with two others also identified previously in human EC cells (TRA-1-60 and TRA-1-81 [4]) appear to become down-regulated during differentiation, a feature which is also observed in hESC cultures exposed to retinoic acid [23, 33]. Although the exact molecular definition of these surface markers remains to be resolved, experiments suggest that they represent carbohydrate modifications, possibly linked to the glycoprotein podocalyxin [51, 64]. The expression of these markers on hESC and their subsequent loss during differentiation suggests a critical functional role in cell pluripotency. Experiments involving the depletion of SSEA-3 and SSEA-4 on hESCs using inhibitors of sphingolipid synthesis appear to show that these markers do not play a critical role in maintenance of the undifferentiated state [13]. Although the function of most of the cell surface markers used for

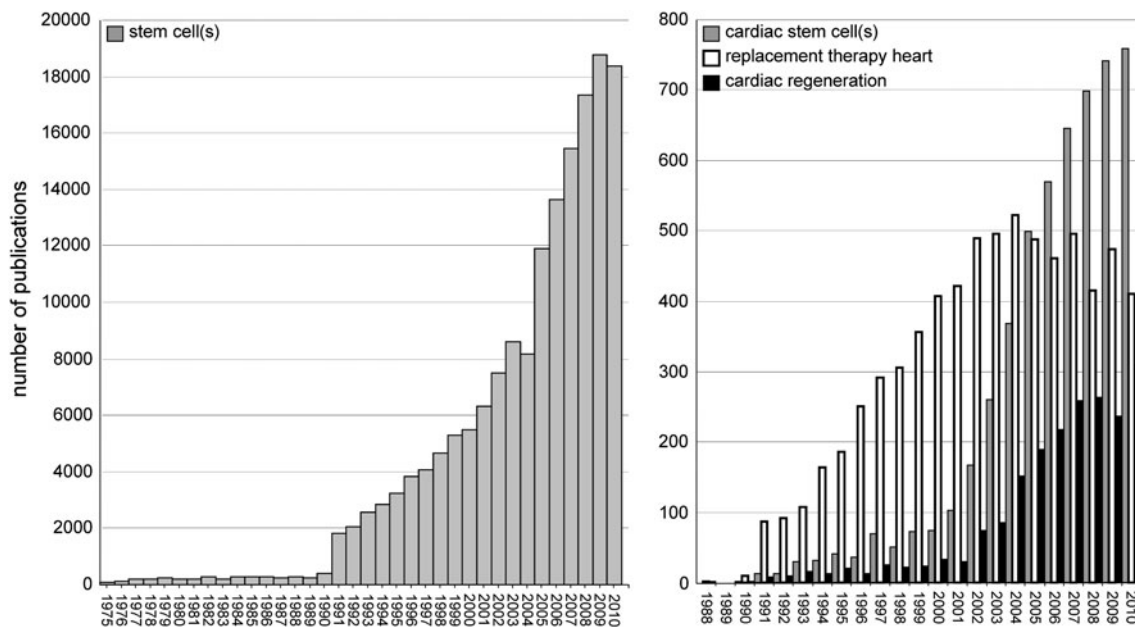


Fig. 1 Annual publication frequency within the field of stem cells and cardiac regeneration

hESC selection remain unknown, the value of identifying unique markers is obvious and remains an area of keen research. For instance, a recent monoclonal antibody screen by Choi et al. [19] has identified a novel surface molecule, E1B-AP5 which co-localizes together with SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81 and becomes down-regulated during differentiation. However, unlike these markers, E1B-AP5's molecular identity is better defined, representing an adenovirus nuclear RNA-binding protein which appears to also be expressed at the plasma membrane as well as in the nucleus of hESC cells.

A further, perhaps more comprehensive, approach to characterizing undifferentiated hESCs has been brought about by the introduction of high-throughput molecular techniques such as microarrays, ChIP-seq and RNA-seq (reviewed in [32]). This work has led to the identification of a core set of transcription factors consisting of OCT3/4 (also known as POU5F1), the sex determining region Y protein SOX2, the homeobox protein NANOG and the PR-domain containing zinc finger protein PRDM14 [18]. These factors are key in the process of pluripotency maintenance, though their individual expression patterns are not all restricted to this earliest phase of development, many being required during later key differentiation and cell lineage decisions. Not coincidentally then, SOX2 and OCT3/4 constitute 2 of the factors used in the initial studies aimed at direct reprogramming of somatic cells to pluripotent stem-like cells (iPS) [67]. The issues and details surrounding iPS cells will be discussed later. In line with this, work from early 2000 demonstrated that a reduction of *Oct3/4* led to a regression of established ES cell lines to a

more trophoblast like state and that an increase in expression of less than two-fold led to differentiation towards the mesodermal and endodermal states [54]. SOX2 has been shown to directly interact with OCT3/4, forming a synergistic regulatory complex regulating many pluripotent stem cell genes. Following on from this Masui et al. [48] have proposed that the major essential function of SOX2 is to stabilize ES pluripotency by maintaining the strict levels of OCT3/4 that a requisite to this undifferentiated state.

In an attempt to try and provide a more comprehensive and systematic view of markers present on hESC lines present in different laboratories around the world, the International Stem Cell Initiative recently characterized 59 hESC lines from 17 laboratories. One of the major conclusions of this study indeed confirmed that the lines were divergent in character; similar expression patterns of all the above mentioned markers were found along with several other unique identifiers [35].

Research surrounding the potential of hESCs for regeneration therapies, although real, has encountered several major obstacles. One of the major, aside from the ethical issues, has been the high risk of teratoma formation. Teratomas are in general benign tumours made up of mixtures of different cell and tissue types caused by the presence of contaminating co-transplanted differentiated cells. This risk was demonstrated to be grounded using animal studies [55]. Although the answer to this potential danger appeared to be pre-differentiation of hESC before injection, following experiments showing the apparent tumour free application of pre-differentiated hESC in animal models, this could not exclude a potential risk if used

in the human clinical setting. This fear may recently have been justified with the first description of a patient treated with multiple injection rounds of foetal neural stem cells for a neurodegenerative disorder developed a multifocal brain tumour [3].

3 Bone marrow mononuclear cells (BMCs)

Although the potential for replacement therapy has received much of its acclaim in field of the embryonic stem cell, perhaps more due to the open ethical discussions surrounding such research, initial studies that recognized the potential of stem cells were focused on haematopoietic and bone marrow derived cells [61]. The relative success and apparent safety of bone marrow replacement therapy, perhaps then contributed to the rapid cardiac clinical setting of this cell source. To this day, BMC has been the most frequently tapped source of cells used in clinical studies and trials aimed at cardiac repair after myocardial infarction.

Typically, BMCs are isolated by aspiration from the iliac crest of the patient themselves, reducing the risks of immunological response in terms of cellular therapy. After an initial phase of processing, often sedimentation or gradient centrifugation, the BMC cell suspension consists of progenitors of endothelial origin (~4%), mesenchymal stem cells (<0.1%) and a very small number of so-called side population cells including a recently identified very small embryonic-like stem cell (VSEL) [70, 84]. The rest of the cellular material, by far the largest portion, is of non-progenitor type [22]. The heterogeneity of the cell population itself and the techniques used for isolation has made the characterization and comparison of sub-populations using cell surface markers difficult. Groups have often selected their own set of markers to define their set of enriched BMC derived cells. The cluster of differentiation (CD) antigens CD14, CD34, CD45 and CD133 [45, 65] have been used to identify and enrich hematopoietic progenitor cell populations (reviewed in [71]). Recently, Riekstina and co-workers examined and determined the presence or absence of a large range of known stem cell markers in an attempt to characterize the mesenchymal progenitor populations from a number of sources including human bone marrow. Like the hESC, the presence of SSEA-4, Oct3/4 and Nanog could be detected in bone marrow derived Mesenchymal Stem Cells (MSCs), though SOX2 appeared to be specifically absent. Further, all sources of MSCs tested appeared positive for the cell surface markers CD73, CD90 and CD105 and lacked expression CD34 [59].

As mentioned earlier, one feature that sets BMC and the sub-derived cell populations has been their rapid implementation in patient clinical trials. Since the 12 month and 18 month data from a large number of these trials has been

discussed and (meta-)analyzed elsewhere [1, 36, 44, 47], we shall only discuss three of the longer-term follow-up clinical trials, though data from such combined analyzes would seem to support the notion of modest improvement in cardiac function after acute myocardial infarction. Most randomized trials have typically only covered a short-term follow-up of 4–12 months, making assessment of the true efficacy and sustained effect of such a therapy difficult. Further, although apparently safe in the short-term application, long-term safety issues have been raised based on reports of associated risks of arrhythmias and calcification [81, 87].

The ASTAMI (Autologous Stem cell Transplantation in Acute Myocardial Infarction) [46], REPAIR-AMI (Reinfusion of Enriched Progenitor Cells and Infarct Remodelling in Acute Myocardial Infarction) [5, 62] and BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration trials) [63] all made use of a randomized controlled patient study in which patients had received, after percutaneous coronary intervention with stenting, an intracoronary injection of BMCs. All three trials found that the application of BMCs in the clinical setting is safe. However, only the REPAIR-AMI (2 years follow-up) [5], but not ASTAMI (3 year follow-up)[11] and BOOST (5 year follow-up) [49] studies, showed a significant positive benefit in left ventricular ejection fraction. This finding can also be concluded from the BOOST 18 month follow-up results and most other clinical BMC trials, with patients typically displaying a 2–3% improvement in left ventricular function. However, after this period, as with the ASTAMI study, BOOST results suggest that this effect steadily declines to become non-significant when compared to control groups. One can perhaps then conclude from these studies that in the long-term there is no observable benefit from the administration of BMCs to patients that have suffered an AMI. However, one should perhaps not be too quick to be dismissive of this cell source. Early phase results in general do see some significant positive effects. Therefore, the lack of information as to why these positive effects disappear during a longer-term follow-up, demonstrates that a more basic approach to understanding the actual mechanisms underlying the observed effects is essential for correct interpretation of the end results. Understanding the underlying mechanism one might be able to focus research and boost the initial as well as sustain these beneficial effects.

One interesting and plausible explanation for at least some of the short-term positive effects observed could be the release of paracrine factors by the injected cell source, which serve to assist recovery of the damaged tissues [15]. Paracrine factors, such as cytokines and growth factors, are often released by cells surrounding areas of tissue damage, functioning as, for instance, circulatory chemoattractants mediating homing of macrophages and stem cells to the

site of injury. Effects may be as diverse as neovascularization associated with release of VEGF and IGF, fibrosis and of particular interest in AMI repair down-regulation thereof (for example HGF [17]) and even cell survival. This may also go some way to explaining why multiple cell sources can have positive effects during the first few months of AMI cellular treatment and recovery. To test this hypothesis, Ziebert and co-workers made use of an inducible thymidine kinase suicide system to deplete transplanted cells in animal models [90]. Induction of this system at specific time points after cell transplantation was observed to reduce the normally observed effects of neovascularization and decrease the level of recovery of left ventricular function and capillary density. Although the paracrine effects in human clinical trials cannot easily be determined, BMC have been shown to release such factors when transplanted into models of ischemia [39, 74].

4 Adipose tissue-derived stem cells (ASC)

Not only is the isolation of bone marrow a rather painful procedure, but also the absolute number of stem cells within the bone marrow is low and their molecular signature is poorly defined. In this respect, adipose tissue could be an attractive alternative source, as it is most often abundantly present in people suffering from cardiac disease, easy to harvest using liposuction, and relatively rich in stem cells. Adipose tissue has been shown to have more than 100-fold higher stem cell density than bone marrow [28]. Adipose tissue gained an interest as a potential cellular source for cardiac regeneration when *in vitro* experiments showed that dispersed adipose tissue can be induced to differentiate into cells of various lineages [91] and even into cardiomyocytes [56]. These experiments revealed that ~0.05% of the plated cells spontaneously differentiated into cardiomyocytes within 20 days of culture. Infusion of non pre-selected or pre-selection of CD44 (hyaluronic acid receptor), CD105 (endoglin) and CD34-negative adipose-derived cells in animal models of acute myocardial infarction, displayed an improved LV-ejection fraction and wall thickness compared to PBS injected controls [8, 16, 75, 89]. In contrast to several studies which apparently demonstrated a loss of infused cells after injection, recent studies using bioluminescent imaging of luciferase transfected adipose-derived cells could be identified in the recipient heart 4 weeks after administration [8]. Histological analysis revealed that the adipose-derived cells had differentiated into cardiomyocytes, smooth muscle cells and endothelial cells in the infarcted region. Moreover, these cells were found to secrete growth factors, like VEGF or IGF-I, possibly contributing to the observed enhanced cardiac function indirectly [7, 8].

Despite several unresolved issues, two double-blind, randomized, placebo-controlled phase I trials, APOLLO and PRECISE, were initiated in 2007; APOLLO, AdiPOSE-derived stem ceLLs in the treatment of patients with ST-elevation myOCardial Infarction, and PRECISE, a randomized clinical trial of adiPOSE-deRived stEm and regenerative Cells in the treatment of patIents with non revaScularizable ischEmic myocardium (<http://clinicaltrials.gov>). The results of which are expected to be presented this year (2011). Nevertheless, at the American Heart Conference in 2010 the 6 month follow-up of the first 14 patients, of which 10 were treated with adipose-derived stem cells and 4 with placebo, were presented. Though the patients treated with adipose-derived stem cells showed a reduction in infarct size, increased myocardial perfusion and ejection fraction, the results did not reach significance most probably due the small sample groups [24].

5 Skeletal myoblast: a direct source of muscle?

Perhaps one of the most obvious sources of cells for cardiac repair are skeletal muscle precursors. These skeletal myoblasts are progenitor cells of adult myofibers with several features which make them an attractive alternative to embryonic and somatic stem cells as replacement cell source; autologous, easy to culture and high scalability *in vitro*, and because they are natural precursors of muscle cells there is a low risk of tumorigenesis. Typically, these cells are isolated from the thigh muscle and then expanded *in vitro* for 3–4 weeks prior to reinjection into the patient. Although early trials (for short review see [25]) demonstrate that transplantation of skeletal myoblasts is feasible and leads to an improvement in heart function in very short-term follow-ups, longer term measurable effects in left ventricular ejection fraction were lost. Clinical trials (MARVEL and SEISMIC), however, are ongoing though results of these trials are scanty, often being announced at meetings or via company press releases (see <http://www.bioheartinc.com>).

Seemingly a perfect source of new muscle, skeletal myoblasts possess one natural inherent barrier that seriously limits their use in directed cardiac cell therapies, namely, unsuitable electrophysiological coupling. Although skeletal myoblasts can be grafted into the heart and can even be sustained for a considerable time, coupling between grafts and resident cardiomyocytes is poor to non-existent leading to the real risk of conduction slowing and re-entrant arrhythmias [31]. One approach to try and overcome this problem is to prevent Cx43 down-regulation, a key gap junction in cardiac conductance, during skeletal myoblast differentiation [20, 58]. Sustained expression of Cx43 in

differentiating skeletal myoblasts appeared to offer improve coupling in model systems, but may not have solved the problem of arrhythmogenicity [27, 60].

6 Deriving cardiomyocytes by programming

The discovery that co-culturing hESCs with endodermal cells led to higher numbers of cardiomyocytes within the differentiating pluripotent population, proved a key initiative in furthering research on cellular replacement strategies [50] reviewed in [80]. This fact, coupled with the finding that transplanted ESCs display an inability to self differentiate into cardiomyocytes in the natural cardiac milieu demonstrated a need for a more basic understanding of signals necessary to induce cardiomyogenesis. The discovery that a combination of at least two TGF β -superfamily members could induce cardiac differentiation in avians and amphibians [78, 79], prompted Laflamme et al. [42] to explore if the same was possible with cultured hESCs. hESCs pre-treated with activin A followed by BMP4 showed yields of >30% beating cardiomyocytes. Later Yang and co-workers adapted and improved this protocol by including a later phase of WNT inhibition via DKK (dickkopf homologue 1) and VEGF (vascular endothelial growth factor) treatment [86]. Similar experiments have also been attempted with BMCs [21, 43].

The advent of techniques to molecularly characterize cells provided a list of potential factors that could drive cardiomyogenesis, but at the same time opened a new door in terms of cellular programming. The possibility of driving a differentiated cell back towards a more pre-differential state began as a list of ESC associated genes up-regulated in the pluripotent state and lost during/after differentiation. The systematic transfection of combinations of these factors finally gave rise to a seemingly magical cocktail of four transcription factors: Oct3/4, Sox2, Klf4 and Myc, which when transfected to adult fibroblasts could derive a stem cell-like state [67]. This technique which has since been shown to induce pluripotent stem cells (iPS) from many different types of fibroblast cells, including human fibroblasts [68] (technique reviewed in [85]). Although the ability to derive cardiomyocytes from this potential new cell source still needs to be fully explored, initial experiments using animal models appear to demonstrate that human fibroblast derived iPS cells can potentially restore function to the damaged myocardium [52].

7 Reprogramming cardiac fibroblasts

Though 90% of the cardiac volume comprises cardiomyocytes, they only constitute 30% of the cells. The vast majority

of the cells in the heart are non-myocytes of which approximately half is cardiac fibroblast [9]. Upon a myocardial infarction the ventricular wall is rescued from rupture by the rapid infiltration of cardiac fibroblasts, healing the wound quickly with simple scar tissue. Although in this respect the cardiac fibroblast is not regenerating the lost myocardium, but replacing it, the prospect of harnessing this naturally abundant cell source and altering its function has been a driving source for recent research into epicardial development and function [77, 79]. A challenging though possible option would be to first induce pluripotency [32, 68] and subsequently differentiate them into cardiomyocytes [69].

Taking fibroblasts as an undifferentiated mesenchymal precursor cell, one could potentially reprogramme this abundant cell source into cardiomyocytes directly, circumventing the necessity to induce a pluripotent state. Zeisberg and co-workers demonstrated that this is a realistic possibility, that transduction of the transcription factor MyoD could induce skeletal muscle differentiation in cardiac fibroblasts. [26]. Following along these lines, Ieda et al. [34] identified key regulators that upon transduction were able to induce myocardial differentiation of cardiac fibroblasts. Making use of isolated GFP-negative cardiac cells from transgenic mice harbouring a GFP driven by alpha-myosin heavy chain promoter transduction of 14 key developmental cardiac regulators induced GFP expression. Sequential removal of single factors revealed that the combination of the transcription factors GATA4, Mef2c and Tbx5 were necessary and sufficient to induce this effect. Further analysis revealed not only the induction of GFP expression, but also the expression of an array of cardiomyocyte specific genes, a genomic methylation status equivalent to cardiomyocytes and spontaneous contractions. Injection of these cells into the left ventricular wall of immunosuppressed mice, revealed GFP-expressing cells at the site of injection. Nevertheless, the used isolation procedures do not exclude that cardiac stem cells are co-isolated. To get a handle on this, the transcription factor cocktail was tested in cKit, Isl1, or Mesp1 depleted cardiac fibroblasts, and remained functional pointing to a direct reprogramming of cardiac fibroblasts rather than inducing cardiac differentiation of co-isolated stem cells. The next challenge will be to test this in human cardiac fibroblast and translate it into an approach that is applicable in vivo in humans.

8 Reflection and future perspectives

The clinical application of progenitor cell sources for cardiac repair rapidly took off with patient trials across Europe and the United States. The promise of an improved cardiac function seemed to be a fact and not just fiction in the initial phases of almost all studies. This spurred large

financial injections from funding bodies and industry. Although even the more sceptical accepted that initial results looked promising, the call for a better understanding of the mechanisms and molecular basis seemed at first to go unheeded as new, better controlled trials, using larger patient groups, were set-up. Questions as to how the infused cell source was inducing positive effects went and remained for a large part, unanswered. Even basic questions surrounding the actual number and type of cells surviving in the infarct after their injection caused a great deal of controversy. As clinical trials progressed and follow-up periods became extended, results began to show a trend change, tending more towards resembling the placebo controls with many of the initial positive benefits of cell therapy being lost over time.

Here, we have tried to present and discuss some of the basic molecular knowledge and clinical trails which attempted to apply the various cell sources in compromised hearts. Although much has been learnt over the past 10 years, many basic scientific questions remain. Many of these questions could be answered by studying nature herself. Understanding how cells develop and differentiate during normal embryogenesis can provide us with many answers to the fundamental questions surrounding this promising area of translational research. One can only hope that the hype within the scientific and medical community, surrounding the first clinical trials and the perspectives of this wonder cure for a growing problem in an aging society, has not destroyed public confidence in this novel area of research. Taken at face value, it would now be easy to dismiss this prospective therapy, just as we are beginning to understand how it could be converted from hope into fact by implementing the mechanistic knowledge that has and is being gained.

A fundamental question that could be posed is why the regenerative capacity of the human heart to repair itself up on damage is virtually non-existing and even insufficient to maintain the number of cardiomyocytes during aging, while lower vertebrates possess the capacity of heart regeneration? Infiltration of fibroblasts into an ischemic area, giving rise to scar tissue, could be considered a kind of ‘quick and dirty’ repair mechanism to prevent heart’s of animals with a high pressure circulatory blood system, (simply) from rupturing after the infarct. If, then, it were possible to assist the damaged heart not only by providing it with a suitable cell source for repair, but also provide a better environment for repair by, for instance, reducing critical hemodynamics and pressures during convalescence, nature may be encouraged to take a more favourable direction in terms of cardiac muscle regeneration.

If it would prove possible to regenerate muscle mass sufficiently, than we should also take into account that heart failure is not simply due to muscle loss. It should be

kept in mind that about half of the patients suffering heart failure do not die due of hemodynamic dysfunction, but rather due to electrical failure and re-infarction. It would, therefore, be prudent to not only focus on the basic mechanisms underlying heart muscle cell regeneration but also to stimulate research into other aspects of heart disease/failure.

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