Which stem cell for cardiomyocyte generation?

A focus on stem cell therapy in heart failure

Bachelorscriptie, Saffloer Mohaupt, September 2010

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Introduction

Heart diseases are the number one cause of death worldwide and the incidence is increasing due to the aging population [1]. The currently used neurohormonal blockade therapies have decreased the mortality rate of heart failure patients [1], but these therapies don't fight the fundamental problems of heart failure and only delay the progression of the disease.

The most recent developments on the properties of stem cells for treating heart failure have caused great excitement in both experimental and clinical research. With the discovery of endogenous stem cells in the human heart, researchers all over the world are looking for a way to use these cells for regenerating the injured heart. Along with other discoveries in the field of stem cells this has lead to optimism that heart failure can be treated with stem cell based therapies. What is the potential of stem cell therapy? On what level is the current knowledge of regeneration in the adult mammalian heart? What are the difficulties in stem cell therapy for treating heart failure? These are some of the questions I want to discuss in this review.

Heart failure: clinical features

Heart failure is a clinical syndrome with a defective cardiac pump function as main feature. There are various conditions that characterize heart failure. For example, mechanical deformities, myocardial abnormalities and rhythmic defects [2]. Heart failure can only be diagnosed when the external symptoms like, dyspnea, fatique and peripheral edema [3] are manifested. Even then it is possible that these nonspecific symptoms are interpreted as a side effect of the administered drug, instead of heart failure. These symptoms can take months to even years to manifest. The asymptomatic period of heart failure patients makes it difficult to register all heart failure patients. This makes it hard to describe the real prevalence and incidence of heart failure. Despite this critical note there are some numbers that give an impression of the prevalence and incidence of heart failure. Mosterd and Hoes [4] stated a prevalence of 1-2 % and a incidence of 5-10 per 1000 persons per year in the Western world.

Heart failure typically occurs in the aging population, i.e. above 50 years of age [4, 5]. Some state that heart failure increases progressively with age [4, 5]. However, this observation could be a result of a progressively increased diagnosis in the ageing population, simply because the symptoms of heart failure become more abundant over the years.

To diagnose heart failure, the European Society of Cardiology guidelines warrant the presence of symptoms and signs, objective evidence of cardiac dysfunction (preferably by echocardiography), and, in case of remaining doubt, a favorable response to treatment directed towards heart failure [4]. For a clearer characterization of heart failure the American Heart Association/American College of Cardiology Foundation created a new classification for heart failure. The classification has 4 stages that are stages A-D. Stages A and B patients are best described as those with risk factors that clearly predispose toward the development of heart failure. Stages C consists of patients which have symptoms of heart failure, most heart failure patients belong to this group. Finally stage D patients have severe heart failure and need specialized treatments.

Heart failure: cardiac remodeling

There are many causes that can lead to heart failure and there are some lifestyle and biological conditions that increase the chance of developing it [5]. Common causes of heart failure are conditions where the heart is ischaemic. Examples that can cause this cardiac ischaemia are: myocardial infarction, ischaemic heart disease, hypertension, valvular heart disease and cardiomyopathy. Irrespective of cause, there are aspects in the

pathophysiology of heart failure that are more or less shared.

A period of cardiac ischaemia will lead to a rapid necrosis of cardiomyocytes, which are the functional pump units of the cardiac muscle. The necrotic cardiomyocytes release reactive oxygen species (ROS) and intracellular proteins that initiate an inflammatory response [6]. The impaired cardiac output, which is due to the loss of cardiomyocytes, triggers the renin-angioten-aldosterone system (RAAS) [7, 8] and the sympathic nervous system. The inflammatory mechanisms, RAAS and sympathic nervous system are a few of the known mechanisms that are activated after cardiac injury. These and other mechanisms try to compensate for the reduced heart function. This complex compensation mechanism consists of processes that remodel the myocardium and processes that modulate vasoconstriction and stroke volume and frequency. For stem cell therapy it is especially important to know the cardiac remodeling that occurs after cardiac injury. Therefore I will only focus on remodeling aspects of the compensation mechanism.

As a result of the loss in cardiomyocytes, the cardiac output decreases. Cardiomyocyte hypertrophy is initiated in order to compensate for increased mechanical load. Hypertrophy is associated with an increase in cell size and protein content, assembly of sarcomeres, activation of a fetal gene program and a decrease of the wall thickness of the myocardium [9]. The cardiomyocytes that are left in the heart are able to increase their work output via these mechanisms. At initial stages cardiac hypertrophy is compensatory, but it is emphasized that it results in heart failure at later stages [10]. It is suggested that a RAAS mediated increase in intracellular Ca^{2+} causes this transition of physiological to pathological hypertrophy [11]. However it is likely that there are other mechanisms involved considering the complex pathophysiology.

An ischaemic episode activates fibrosis mechanisms; sites of previous cardiomyocytes necrosis are replaced with scar tissue to preserve structural integrity [12]. With extensive cardiomyocytes loss, the scar tissue can be widespread. Scar tissue consists predominantly of stiff type I fibrillar collagen, this together with the cardiomyocytes loss contributes to the progressive failure of the heart [13].

Besides hypertrophy and fibrosis, there are two other aspects in the cardiac remodeling that occur and impair heart function. One of these is the accumulation of extracellular matrix. The extracellular space of the myocardium consists of nonmyocyte cells of which >90% are fibroblasts. The fibroblasts will enhance their collagen synthesis in response to the cardiac injury. The stiff collagen fibers lead to further cardiac dysfunction [14]. There is accumulating evidence that Aldosterone, a end product in the RAAS, plays an important role in the cardiac collagen deposition [13, 14].

Another aspect in the cardiac remodeling is the inadequate development of coronary vasculature. In some cases the coronary vasculature was already impaired and probably cause of the ischeamic episode. However, for the development of functional heart tissue it is crucial to have a sufficient supply of blood.

To summarize, extensive collagen deposition, cardiomyocytes hypertrophy and impaired blood supply all form a complex network that complement each other in the progression of heart failure.

Current treatments for heart failure

Because of the high mortality, morbidity and costs of heart failure, it is essential to understand the pathophysiology of this syndrome. With that knowledge it may be possible to develop therapies for heart failure. Research has lead to different therapeutic targets. Most of the current treatments for heart failure are based on pharmaceuticals. These treatments only affect the symptoms. The cardiac remodeling that underlies heart failure is left untreated. The currently used treatments are: (RAAS) blockade, Bèta-blockers and heart transplantation.

RAAS blockade

The pharmaceuticals that are currently used inhibit the renin-angioten-aldosterone system (RAAS) cascade at different levels [2]. This group of RAAS interfering pharmaceuticals can be subdivided into: ACE inhibitors, Ang.II receptor type 1 antagonists and aldosterone antagonists. RAAS blockade results in a decrease of the RAAS mediated vasoconstriction and cardiac remodeling, which can partially improve the cardiac function of heart failure patients [2, 7, 15].

Beta-blockers

Activation of the sympathic nervous system causes a stimulation of the β-adrenoceptors on heart tissue, which will increase the contractile force of the heart tissue, leading to an increase in blood pressure. Beta-blockers will block these β-adrenoceptors and the effect of this is a lower blood pressure. It doesn't seem obvious to use beta-blockers by a heart failure patient, because the cardiac output is already decreased and the use of beta-blockers will only decrease this more. However with the progression of the disease increased sympathetic drive causes cardiotoxic effects via cardiac and subcellular remodeling with LV dysfunction, cardiomyocyte death, defects in Ca2+-cycling proteins and attenuated contractility [2]. In these stages of heart failure beta-blocker therapy seems to be beneficial. [2, 16, 17].

Heart transplantation

Replacement of the patient's heart by a donor heart is the most effective approach for end-stage heart failure.

Life-long immunosuppressant administration, the lack of sufficient donor hearts and operative complications are the main problems of this procedure. However, transplantation will extend the life time of the patients compared to natural course of the syndrome. On average there is a 10 years survival rate of 50% of the patients that receive a donor heart [18].

Intrinsic cardiac regeneration

As with all treatments, the treatments mentioned above, are all unnatural. Does the heart have intrinsic mechanisms to cope with heart failure? And if there are mechanisms available, what is their share in restoring heart function?

Since last decade it has been established that the human heart has some self renewal capabilities. Kajstura et al demonstrated in 1998 the replication of cardiomyocytes in end-stage heart failure [19]. In addition, Quaini et al showed that 11 nuclei per 1 million cardiomyocytes exhibited mitotic figures in chronic heart failure patients [20]. These studies demonstrate that the heart is not completely post-mitotic. However, the intrinsic regenerative mechanisms are way too inadequate to compensate for the severe loss of cardiomyocytes that precedes heart failure [21].

Both the limited regenerative capacity of the human heart and the unsatisfactory results of the current treatments for heart failure have developed a drive to find new satisfactory methods to treat heart failure. One of the promising and exciting methods is stem cell therapy. New types of stem cells are found and methods are discovered that make it possible to

turn somatic cells into 'induced' stem cells. To investigate the potential of regenerative stem cell therapy for the treatment of heart failure, it is essential to know the classification of the different stem cells and what research has been done with stem cells in the context of heart failure.

Classification of stem cells

Stem cells are undifferentiated cells that can be found in every multi-cellular organism. The classical definition of a stem cell requires that it possesses two main properties: 1) self-renewal and 2) potency. Self-renewal is the ability of a stem cell to divide while maintaining the undifferentiated state. The cell divisions that are essential for self-renewal can be categorized into two types: symmetric divisions, where the two daughter cells are complete stem cells and there are asymmetric divisions where only one of the two daughter cells is a complete stem cell, the other daughter cell is called a progenitor cell and has limited self-renewal potential. Progenitor cells can go through a couple of cycles of cell divisions before they differentiate. This number of cycles is marginal compared to complete stem cells. Potency is the second property that is required to classify a cell as a stem cell. In this context potency refers to the potential of a cell to differentiate into different cell types. There are different degrees of potency. However, two forms of potency are relevant for the focus of this review, namely pluripotency and multipotency.

Pluripotent stem cells

Pluripotent cells can differentiate into all derivates of the three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm) [22].

-Embryonic stem cells-

The best known pluripotent stem cells, is the embryonic stem cell. Embryonic stem cells (ESCs) usually refer to the inner cells of a 3- to 5-day-old embryo, called a blastocyst [22]. Under specific conditions these pluripotent cells can differentiate into every cell type [22].

The research of embryonic stem cell lines has generated much interest and debate. This because the ESCs are isolated from human embryos that are only a few days old. The question that rises: is it ethically correct to sacrifice human embryos for (medical) research? Opinions about this question differ and are hard to characterize, because of the complexity and the sometimes contradictory views [23]. Nevertheless, the ethical concerns have impacted both the public opinions and the availability of public monies in a mostly disadvantuous manner. Furthermore, ESCs carry the risk of teratoma formation [22, 24] and immune rejection. A teratoma is an encapsulated tumor with tissue or organ components resembling normal derivates of all three germ layers.

The combination of ethical problems, the increased risk of teratoma formation and immune rejections make ESCs an inferior candidate for regenerative therapies compared to somatic stem cells and therefore they will not be further discussed in this review.

-Induced pluripotent stem cells-

ESCs are pluripotent from origin. However, in 2006 Takahashi and Yamanaka [25] identified conditions that could transform adult differentiated somatic cells into cells with stem cell like abilities. These reprogrammed somatic cells are called 'induced pluripotent stem (iPS) cells. The discovery of reprogramming somatic cell generated great excitement, because differentiated cell were transformed into pluripotent cells with only the introduction of a few genes [25]. Considering the regenerative opportunities, iPS cells seem to have relevant features. First iPS cells are pluripotent so they can form any cell type of the body; second they offer prospect of producing patient specific cells,

thereby avoiding immunorejection; and third iPS cells circumvent the ethical problems surrounding the use of human embryonic stem cells.

In the initial study of Takahashi and Yamanaka [25], somatic cells were reprogrammed by the retroviral transfection of just four genes. The genes Sox2, Oct4, Klf4 and c-Myc (SOKM) were sufficient for reprogramming to occur [25]. After the discovery of iPS cells several refinements in the reprogramming protocols have been made, to improve the speed and efficiency. This has revealed that there is some flexibility in the factors needed for reprogramming somatic cells. Human fibroblasts, for example, have been reprogrammed using SOKM [26] as well as Sox2, Oct4, Lin28 and Nanog (SOLN) [27]. However, in both groups, the reprogramming genes were transfected with the use of a retrovirus. When genes are transfected with retroviruses they permanently integrate into the host's genome, to express the reprogramming genes. This can have devastating side effects like continued expression or reactivation of the reprogramming genes, which can lead to tumor formation [27] or it can influence cell differentiation [28]. To minimize the risk of unwanted expression of the retroviral transfected reprogramming genes, several methods were developed. For example, reprogramming factors were integrated into the host genome by retroviruses and after reprogramming the factors were removed from the genome using Cre-lox recombination [29]. Other researchers used adenoviruses instead of retroviruses, because adenoviruses do not integrate into the host's genome [30]. Recent studies show that the transfection of genes is not required for reprogramming somatic cells: In physiological cellular conditions pluripotency and differentiation is inhibited by histone acetylation and DNA methylation at the promoter regions of the the following genes: OCT4, SOX2 and Nanog. Cells treated with 5-azacytidine and/or valproic acid lose these inhibitions through inhibition of acetylation or DNA demethylation [31]. After this process different external and internal factors can be used to modulate the pluripotency and differentiation of the induced stem cells.

In most mechanisms, that are used to reprogram somatic cells, a modification of the cell's DNA sequence or structure is required. Multiple inducing factors that are used in these 'inducing' mechanisms have oncogenic potential [32]. Moreover, the mechanisms can activate cancer-specific promoters and can cause cancer-specific epigenetic changes [33]. And because iPS cells are pluripotent, just like ESCs, they have the potential of forming teratomas [34, 35]. A recent study showed that the frequency of teratoma formation is even higher in mice transplanted with iPS cells compared to mice transplanted with hESCs (100% vs. 88% respectively) [34]. These types of uncontrollable manifestations are inacceptable when considering iPS cells for clinical relevant regenerative therapies.

Multipotent progenitor cells

A form of potency that is less advanced than pluripotency is multipotency. The progenitor cells, which are a result of asymmetrical cell divisions, belong to this category. Multipotency means that a cell is able to differentiate into a limited number of cell types. The differentiation routes that are available depend on the origin of the multipotent progenitor cell.

-Adult progenitor cells-

Adult progenitor cells, also referred to as somatic progenitor cells, are undifferentiated, post-embryonic progenitor cells that are identified in different types of tissues, i.e., bone marrow, epidermis, central nervous system, blood vessels, skeletal muscle, skin, gut and liver [36]. Adult progenitor cells reside in a specific niche in a tissue or organ. In this niche they remain non-dividing until they are needed for normal maintenance or for tissue repair due to disease or injury [36]. Most adult progenitor cells are multipotent and can differentiate to yield some or all of the cell types of

the tissue or organ [37]. Generally they are referred to by their tissue of origin (like bone marrow derived progenitor cells, neural progenitor cells etc.). Then there exist pluripotent adult stem cells, which are very rare. To give an indication of the rarity of pluripotent stem cells: 1 of the 10^7 to 10^8 cells in the bone marrow is a pluripotent bone marrow cell [37]. This makes it difficult to isolate them. Often, preparations of pluripotent adult stem cells are contaminated with multipotent adult progenitor cells.

First it was thought that adult progenitor cells were only able to generate specialized cell types of tissues form which they normally reside. It is now generally accepted that some adult progenitor cells can differentiate into other cell types that lie outside their tissue of origin [38]. This phenomenon is called 'plasticity'. Despite the plasticity of adult progenitor cells, they remain less potential than embryonic stem cells. Embryonic stem cells have the ability to differentiate into all cell types, adult progenitor cells have to deal with a more limited cell type spectrum. The fact that most adult progenitor cells are not pluripotent has as advantage that they are not inclined to develop teratomas [35, 39]. Neither are adult progenitor cells ethically debated nor immunologically rejected.

-Cardiac progenitor cells-

Cardiac progenitor cells (CSCs) are populations of resident progenitor cells in the human myocardium that have been discovered recently [40, 41]. This type of multipotent stem cell has the potential to differentiate into cardiomyocytes, endothelial and vascular smooth muscle cells [40, 41]. The proliferation and differentiation potential have been demonstrated both *in vitro* and *in vivo* [42]. Understanding the mechanisms of differentiation would offer the opportunity to potentiate this process and promote cardiac repair. Besides their therapeutic implications, these observations strengthen the view that the heart is not a postmitotic organ.

Progenitor cells in regenerative cardio-vascular medicine

Due to their unique regenerative features, stem cells have potentials for treating cell based diseases, such as heart failure. However to use stem cells to their full potential, knowledge about their intrinsic mechanisms, signaling and dividing is essential. Much work has been done and research on stem cells continues to advance. In the next paragraph I will give an overview of what is known about the use of stem cells in regenerative cardiovascular medicine.

Induced pluripotent stem cells

Mauriz *et al.*[43] investigated the potential of iPS cells to differentiate into myocardium relevant cells types. They demonstrated that iPS cells from mice were capable of forming functional contracting cardiomyocytes that showed the same typical features of ESC-derived cardiomyocytes. Yokoo *et al.*[44] showed that it was possible to generate functional contractile cardiomyocytes from human iPS cells. The engineered cardiomyocytes reacted the same on cardioactve drugs as cardiomyocytes derived from ESC, or human cardiomyocytes in a clinical setting. Recently Leda *et al.* [45] showed a direct reprogramming of cardiac and dermal fibroblasts into cardiomyocytes. In this procedure a retroviral transfection of cardiac fibroblasts with three factors (Gata4, Mef2c and Tbx5) resulted in direct differentiation into cardiomyocytes. Because the pluripotent state op iPS cells is passed in this procedure, it might be possible that there will be a decrease in the chance of developing teratomas. However, this was not reported in the journal.

Adult progenitor cells

Goodell et al. [46] showed that bone marrow derived progenitor cells can contribute to cardiac muscle repair and neovascularization after ischemic heart injury. The engrafted progenitor cells differentiated into cardiomyocytes and endothelial cells and contributed to the formation of functional

heart tissue. This observation seems like an expansion of the opportunities that adult progenitor cells can have in regenerative therapies for heart failure, however it must be taken into account that these positive results are obtained in a specific experimental setup. More recent studies, that investigate if adult progenitor cells can contribute to the regeneration of the infracted myocardium, show less hopeful results [47, 48]. For example, Martin-Rendon *et al.* [47] investigated the potential of mesenchymal progenitor cells (MPCs) to generate cardiomyocytes *in vitro*. They used MPCs from different sources (perivascular tissue, umbilical cord, cord blood and from bone marrow), nevertheless only the bone marrow derived MPCs were capable of forming cardiomyocytes-like cells at a very slow rate (approximately 0.07%). These results show that MPCs are not capable of forming a relevant number of cardiomyocytes for cardiac repair. An *in vivo* study of Agbulut *et al.* [48] in coronary ligated rats demonstrated that a subpopulation of rat bone marrow derived adult progenitor cells were neither capable to differentiate into cardiomyocytes nor to engraft into the heart.

The data of both *in vitro* and *in vivo* preclinical studies suggest that it is unlikely that differentiation of bone marrow-derived adult progenitor cells into cardiomyocytes happens to any relevant degree. Although there are some studies that report successful transdifferentiation, most studies report insignificant results. Despite these observations different types of bone marrow-derived progenitor cells have been found as potential sources for cell therapy of heart failure [49, 50]. However, it is now generally accepted that transplanted bone marrow-derived progenitor cells exert their beneficial role through paracrine effects on structural (ventricular remodeling, vascularisation) and functional (heart ouput) parameters [51, 52].

At present, the most clinical experience has been obtained with the use of adult progenitor cells. A clinical trial using bone marrow-derived cells have reported positive effects on left ventricular function, tissue perfusion and the patients' viability [53]. There are some other studies that report an increase in cardiac function after the administration of autologus bone marrow derived cells [54]. It is unknown what the contribution of the paracrine effect is on these promising results.

Recently the Cardiovascular Cell Therapy Research Network (CCTRN) started the design FOCUS (First Mononuclear Cells injected in the US). This will be a randomized, phase II, placebo-controlled clinical trial that will assess the effects of autologous bone marrow mononuclear cells delivered trans endocardially to patients with left ventricular (LV) dysfunction and symptomatic heart failure or angina [55]. This study will probably reveal if bone marrow mononuclear cells can have a beneficial role in the threat of heart failure.

Cardiac stem cells

It has been hypothesized that stimulation of the intrinsic repair mechanisms of the heart could be a promising approach to promote cardiac regeneration and thereby improving heart function. Rota et al showed that an injection of cardiac stem cells with insulin-like growth factor-1 activated resident cardiac stem cells, can replace approximately 42% of cardiac scar tissue that was formed after a heart infarct [56].

Besides intrinsic stimulation, Bearzi *et al.* [42] showed that hCSCs derived from small samples of the human myocardium can be isolated and expanded *in vitro*. Moreover, these hCSCs differentiated predominantly into cardiomyocytes and, after local injection in infracted myocardium of immunodeficient mice, they structurally and functionally integrated into the rodent myocardium.

Thus far there are no clinical trials that describe the results of CSCs in regenerative therapies for humans, but the use of it will be free from the ethical debates associated with the use of hESCs and the risk of immune rejection will, most likely, be absent.

Conclusion

All conventional treatment options that are clinically used for heart failure lead to a moderate improvement in either the patient's mortality or morbidity. These unsatisfactory results are caused by the way that conventional treatments work: they reduce the symptoms mainly by inhibiting the RAAS and sympathic nervous system. These systems are, among others, involved in cardiac remodeling and vasoconstriction. However, despite the inhibition of these mechanisms, the cardiac remodeling progresses. Fibrosis, hypertrophy, extracellular matrix accumulation and inflammation are all part of this cardiac remodeling that is initiated to compensate for the loss in cardiomyocytes. The heart's physiology changes dramatically due to these processes. Cardiomyocytes are replaced with stiff scar tissue, the remaining cardiomyocytes are hypertrophied and surrounded with a stiff extracellular matrix. Therefore it is unrealistic to think, that heart failure can be cured with the supply of cardiomyocytes generated out of stem cells. These cardiomyocytes need to successfully incorporate in the remodeled heart, although presence of scar tissue, collagenous extracellular matrix and lack of adequate vascularization are limiting this.

This makes the treatments of heart failure bilateral. 1) There is the modeled heart, that impairs successful integration of 'new' cardiomyocytes. 2) Generation of 'new' functional cardiomyocytes that can replace the ones that were lost as a result of necrosis.

For the first part it is essential to know the pathways that are involved in cardiac remodeling. Inhibiting the cardiac remodeling might be a solution to extend the time frame for successful integration of 'new' cardiomyocytes. Additionally, Heart failure has a large therapeutic window; it might be possible that with early diagnosis of heart failure, stem cell therapies can be applied before the cardiac remodeling.

The second part is the creation of functional and structural correct and save cardiomyocytes. The field of stem cell therapy for is growing. New strategies based on developments in isolation, manipulation and survival enhancement are created. This all is true for the research in stem cell therapies aimed at the treatment of heart failure. The most obvious, but difficult to answer, question is: which type of stem cells is the best candidate for heart failure therapy?

Adult progenitor cells are thus far the most studied type of progenitor cell. They are harvested from the patient in whom they are ultimately to be used. Therefore they do not need to overcome an immunologic and ethical barrier. These features make them good candidates for regenerative therapies. However, the differentiation potential is controversial because they are already lineage committed and their replicative capacity is limited [57].

ESCs are capable of producing cardiomyocytes [58, 59], but are ethically controversial, legally bordered, have the greatest risk for forming teratomas and immunological rejection. This makes them inferior candidates compared to the other stem cell types.

CSCs are recently discovered, so isolation and manipulation procedure are in early development stages. The advantages of CSCs are that they are patient specific and are capable of forming cardiomyocytes. However, in heart failure patients the CSCs are often rare or dysfunctional [57]. This means that the patient specific aspect of CSCs cannot apply, simply because the CSCs are lost or damaged during, for example, the ischaemic episode.

IPS cells have a lot of advantageous features: first, they can be harvested from the patient, which eliminated the risk of immune rejection. Second they can differentiate into cells from all embryonic germ layers; third, iPS cells are ethically not controversial. The main disadvantage of iPS cells is

the risk of forming teratomas and tumors. Teratoma formation is typical for pluripotent stem cells [60]. Therefore it is also observed with ESCs. This feature of pluripotent stem cells is a main drawback for using the in stem cell based regenerative therapies. However, with the discovery of a procedure that makes it possible to differentiate cardiac fibroblast directly into cardiomyocytes [45], it might be possible that the risk on teratoma formation is reduced. Another advantage of this procedure could be the *in vivo* conversion of cardiac fibroblast into cardiomyocytes. This could be of great potential in heart failure patients, because the fibroblasts that are contributing to cardiac remodeling could be converted into functional caradiomyocytes. This application is hypothetical, but investigating the possibilities could be interesting.

To answer the question: which type of stem cells is the best candidate for heart failure therapy? Adult progenitor cells and the recently discovered procedure to directly reprogram cardiac fibroblast are good candidates. But even if we know the 'best stem cell' for the treatment of heart failure, then there are other crucial questions to ask. For example, what is the best way to deliver the stem cells? How can the transplanted stem cells survive and integrate in the remodeled heart? These are just a few of the questions that need to be answered in this exciting field of research.

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