REVIEW

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Bringing cardiac stem cell therapy from bench to bedside: lessons from the past and future perspectives

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Abstract

Findings in the cardiology field from the last three decades of the 20th century were ruled by the theory that the heart is a post-mitotic organ, incapable to regenerate. Recent studies have brought evidences regarding the existence of some cells residing in the adult heart, having stem properties. These cardiac stem cells (CSCs) govern myocardial homeostasis and repair by differentiating into new cardiomyocytes, smooth muscle cells and vascular endothelial cells and also by releasing proangiogenic and procardiogenic cytokines. Hence, CSC-based therapy seems to be a promising tool for repairing failing hearts. This review presents the current data regarding various subpopulations of CSCs and their regenerative potential revealed by phase I clinical trials; finally, future perspectives for the development of more advanced therapeutic protocols are proposed.

Keywords: cardiac stem cells, cardiospheres, cardiac regeneration.

Introduction

Findings in the cardiology field from the last three decades of the 20th century were ruled by the theory that the heart is a post-mitotic organ, incapable to regenerate [1]. This theory was based on two facts: adult cardiomyocytes are terminally differentiated cells that are unable to reenter the cell cycle and, respectively, the lack of stem cells (SCs) in the myocardium. From birth until old age, the myocardial growth was believed to be based on hypertrophy, and in the absence of any cardiac disease, it was supposed that the number of cardiomyocytes remained constant for the whole life. The '90s brought proofs that infirmed this theory, by showing the fact that adult mammals' cardiac myocytes are capable to reenter the cell cycle, and furthermore, they are able to participate to karyokinesis and cytokinesis [2, 3]. Even under normal circumstances, the number of myocardial cells that dies grows proportional with the age; until middle age this cellular loss is over-countered through formation of new cardiomyocytes, but eventually it shows the reduction of their number, which tries to be countervailed by the hypertrophy of the remaining cells. Retrospective studies realized with the ^{14}C isotope have showed that during the life of an individual approximately 50% of the cardiomyocytes are renewing, thus indicating the existence of a natural system of repair, which varies with the age, but inadequate in the face of a major ischemic event [4, 5].

The existence of postnatal cardiogenesis was confirmed by future studies using animal [6–8] or human models [9], all showing that cardiac regeneration is reducing with ageing. From this perspective, the cardiac homeostasis presumes a balance among cellular death, regeneration and hypertrophy.

Adult resident CSC populations

The beginning of the 21st century has brought evidence regarding the existence of some cells residing in the heart, having stem properties; depending of the expressed markers, different research groups have described more types of cardiac stem cells (CSCs) (c-kit+, Sca-1+, Isl1+, cells named "side population"); although, it is not excluded that different cellular types are only the phenotypic expression of the same cell (which is able to express markers in different combinations), or that the reported differences are due to isolation and cultivation methods followed in various labs (Table 1 shows the markers expressed by cardiac stem cell and the authors that described them – adapted after [10–13]).

Table 1 – ***Cellular markers expressed by CSCs***

Markers		References
CD177/c-kit	+	Beltrami *et al.*, 2003 [14]; Matsuura *et al.*, 2004 [15]; Messina *et al.*, 2004 [16]; Anversa *et al.*, 2006 [17]; Di Felice *et al.*, 2007 [10]; Dey *et al.*, 2013 [11]; Ellison *et al.*, 2013 [12].
	-	Oh *et al.*, 2003 [18].
Sca-1	+	Oh *et al.*, 2003 [18]; Matsuura *et al.*, 2004 [15]; Messina *et al.*, 2004 [16]; Anversa *et al.*, 2006 [17]; Di Felice *et al.*, 2007 [10]; Dey *et al.*, 2013 [11].
MDR-1	+	Quaini *et al.*, 2002 [19]; Anversa *et al.*, 2006 [17].
CD31	+	Oh *et al.*, 2003 [18].
CD34	-	Oh *et al.*, 2003 [18]; Di Felice *et al.*, 2007 [10].
	+	Matsuura *et al.*, 2003 [15]; Messina *et al.*, 2004 [16].
CD38	+	Oh *et al.*, 2003 [18].
CD45	-	Oh *et al.*, 2003 [18]; Di Felice *et al.*, 2007 [10].
	+	Matsuura *et al.*, 2004 [15].
Cardiac troponins	+	Matsuura *et al.*, 2004 [15]; Messina *et al.*, 2004 [16]; Di Felice *et al.*, 2007 [10].
Cardiac actin	+	Beltrami *et al.*, 2003 [14]; Matsuura *et al.*, 2004 [15]; Di Felice *et al.*, 2007 [10].
Tropomyosin	+	Matsuura *et al.*, 2004 [15].
α- or *β*-Heavy chain of cardiac myosin	+	Beltrami *et al.*, 2003 [14]; Matsuura *et al.*, 2004 [15]; Messina *et al.*, 2004 [16]; Laugwitz *et al.*, 2005 [20]; Di Felice *et al.*, 2007 [10].

Markers		References
Smooth-muscle actin	+	Beltrami *et al.*, 2003 [14]; Di Felice *et al.*, 2007 [10].
Connexin 43	+	Matsuura *et al.*, 2004 [15]; Di Felice *et al.*, 2007 [10].
MEF-2C	+	Beltrami *et al.*, 2003 [14]; Oh *et al.*, 2003 [18]; Matsuura *et al.*, 2004 [15].
Nestin	+	Quaini *et al.*, 2002 [19]; Di Felice *et al.*, 2007 [10].
GATA-4	+	Beltrami *et al.*, 2003 [14]; Oh *et al.*, 2003 [18]; Matsuura *et al.*, 2004 [15]; Di Felice *et al.*, 2007 [10].
Csx/Nkx-2.5	+	Beltrami *et al.*, 2003 [14]; Matsuura *et al.*, 2004 [15].
Isl-1	+	Laugwitz *et al.*, 2005 [20]; Di Felice *et al.*, 2007 [10].
vWF	-	Beltrami *et al.*, 2003 [14].
Abcg-2	+	Martin *et al.*, 2004 [21].

CSCs: Cardiac stem cells; CD: Cluster of differentiation; MDR1: Multi-drug resistance; MEF-2C: Myocyte-specific enhancer factor 2C; GATA-4: GATA binding protein 4; Isl-1: Islet 1; vWF: von Willebrand factor; Abcg-2: ATP-binding cassette superfamily G member 2.

First to describe the presence of such cells was Beltrami's group in 2003, which identified in the rat myocardium a lin-/c-kit+ cell population with clonogenic proprieties, capable of generating – *in vitro* – both cardiomyocytes and vascular cells [14]. In the adult heart, the activation of CSCs Oct+ induces symmetric, but also asymmetric division, generating c-kit+ cells, which will differentiate into myocyte (GATA-4, Nkx-2.5, MEF-2C, α-sarcomeric actin), endothelial (Ets-1, von Willebrand factor) and smooth muscular line (GATA-6, actin). Differentiated cells, even the small cardiomyocytes, continue to proliferate, fact confirmed by bromodeoxyuridine incorporation of DNA and the expression of Ki67 nuclear protein; thus, CSCs are multi-potent, clonogenic and capable of self-renewal [22].

The studies conducted on animal models showed the feasibility and benefits of c-kit+ cells administration on ventricular function, remodeling and dimension of myocardial necrosis [23]; existence of this type of cells was described also in the human heart, the generation of new cardiomyocytes being shown in the case of patients with aortic stenosis and ischemic cardiomyopathy [24, 25].

Another population of cardiac progenitor cells residing in the mouse heart is called Sca-1+. *In vitro*, these cells differentiate in cardiomyocytes, being CD31+, but c-kit-; in the presence of oxytocin, they express proteins and cardiac transcription factors, like GATA4, cardiac actin, troponin T, tropomyosin, connexin 43; injected intravenously in mice with myocardial infarction, Sca-1+ cells generate cardiomyocytes, with mild effects on myocardial regeneration.

Another type of CSC is that expressing the transcription factor Islet 1 (Isl-1+) [20]; it is a well-known fact that Isl-1 is showed in cells involved in murine cardiac morphogenesis during embryonic life, while the homozygote deletion of this factor leads to defects in development of the right ventricle (RV), atria and outflow tract of the RV, but without affecting the development of the left ventricle (LV). Regarding this data, Anversa *et al.* stipulated that the presence of Isl-1 does not define a particular type of CSC, but is attributed to the start of differentiation to myocyte line [17].

CSCs, which are part of side population (SP) subgroup, are defined by the capacity to exclude Hoechst 33342 and Rhodamine 123 dyes, due to the rapid efflux mediated by the ATP-binding cassette superfamily G member 2 (ABCG2) and multidrug resistance (MDR1) transporters [26].

Anversa *et al.* have proposed to classify the immature cardiac cells in four groups, from the primitive to the differentiated ones: CSCs, progenitors, precursors and amplified cells; first three types express c-kit, Sca-1 and the multi-drug resistance factor [17]. This theory is asserted by recent studies that have shown the fact that c-kit+ cells are undifferentiated primitive cells, while the CSCs Sca1+ with transcriptional profile are the closest to cardiomyocytes; both SP type cells and Sca1+ cells express on their surface the Sca1+ antigen, while being c-kit-, which led the authors to state that those two cellular types represent different stages of the same progenitor cell [11].

CSCs are distributed patchy in the heart; although the majority of CSCs are located in the LV, the higher concentration on the volume unit is in those areas of the heart with low parietal stress, such as the atria and the apex, where the mean concentration of this cells in viable myocardium is approximately one CSC/10 000 cardiomyocytes (similar percentage with hematopoietic stem cells in bone marrow), but with great variation between the results published by different research groups.

The studies realized on mice [15, 25], rats [14] and humans [25] have indicated the existence of one CSC to 8000–20 000 myocytes, or even two CSCs/25 myocytes [18, 21], but probably these high values have also included the presence of endothelial progenitors.

At myocardial level, CSCs are nested in structural and functional units called niches; the CSCs niche has an essential role in determination of cellular behavior under physiological or pathological circumstances in regard to maintaining the undifferentiated character, and also the growth, migration and differentiation of these cells. *In vivo*, the CSCs niche is subepicardial located, near the coronary arteries. A central feature of the niche is represented by telocytes, cells that, through thin and long prolongations, make contact with the CSCs and the cardiac progenitor cells, having a supportive and informational purpose [27, 28].

Cardiosphere-derived cells

Myocardial regeneration respects a well-known procedure, which states that CSCs generate cellular precursors restricted to a certain cell line, which in the end turn into terminally differentiated cells. Some studies showed that harvested CSCs from murine and human myocardium are able to be grown as spherical aggregates called cardiospheres; *in vitro* architecture of cardiospheres mimicks that of *in vivo* CSCs niche.

In 2004, Messina's group obtained cardiospheres from murine and human cells harvested through myocardial biopsy at atrial and ventricular site [16]. Grown in Petri dishes, these cells do not adhere, but form three-dimensional aggregates named cardiospheres, which are similar to embryoid bodies and neurospheres. Cardiospheres express endothelial markers (human: KDR, mice: flk), but also stem markers (CD34, c-kit, Sca-1); each cardio-

sphere is the result of proliferation of a single cell, and is capable of generating cardiomyocytes, smooth muscle cells and endothelial cells. Thus, the center of this structure is made of c-kit+ primitive cells, surrounded differentiated cells expressing myocyte proteins and connexin 43 arranged in successive arrays, while at periphery a layer of mesenchymal stromal cells can be found. Connexin 43 has a dual function: in undifferentiated progenitors it stimulates proliferation, whereas in differentiated cells towards cardiomyocyte line it facilitates the electric coupling with surrounding cells. Undifferentiated cells are connected with differentiated ones through gap junctions. Unlike monolayer classic-cultivated CSCs, cardiospheres contain a higher percentage of c-kit+ cells, enhanced expression of the genes involved in cellular renewal (Nanog and SOX2), as well as augmentation of some growth factors and extracellular matrix molecules specific to stem cells: insulin-like growth factor 1 (IGF-1), α2 integrin, β1 laminin; dissociation of cardiospheres into single cells decreased the expression of adhesion molecules and the resistance of cells to oxidative stress [29].

Murine cardiospheres derived cells proved to have spontaneous contractile activity, while the human ones became contractile only after 24 hours of cultivation in the presence of rat cardiomyocytes; *in vivo*, on a murine model, it has been shown that these cells contributed to myocardial regeneration [16].

Later on, in 2007, Smith *et al.* used the technique described by Messina in order to obtain cardiospheres from human percutaneous endomyocardial biopsy specimens; acquired cells were proven to be positive for c-kit, but not for MDR1. Similar to Messina's findings, they did not have spontaneous contractile activity, but gained a synchronized action potential after co-cultivation with newborn rat cardiomyocytes [30].

Transplanted in infarcted myocardium, CSCs – as well as cardiospheres-derived stem cells (CSCd) – differentiate and release proangiogenic and procardiogenic cytokines, thus restricting the cardiac remodeling, improving the function and perfusion in necrosis-affected areas in both direct and indirect (paracrine) manner [14, 23, 31–35].

Growth conditions influence decisively the effects of transplanted cells on the cardiac function. Transplantation of CSCd (derived from first or second generations of cardiospheres) was proven to be more effective than administration of cells derived directly from biopsy explants. Three weeks after CSCd transplantation, LV ejection fraction (LVEF) improved to 37.7±4.6%, respectively 38.2±3.2%, while in mice receiving cells derived directly from biopsy explants mean LVEF was only 32.1±3.3%. Still, comparing to control animals recording degradation of systolic function (LVEF 23.5±2.6% at three weeks follow-up *versus* 29.3±3.2% at the study beginning), the administration of cells derived directly from biopsy specimens had a benefic effect, even though of lower amplitude. This data emphasize the importance of the three-dimensional architecture in maintaining the cells in an undifferentiated state [36].

Another key determining factor of transplanted cells fate, as well as of cardiac benefit, is the capacity of transplanted cells to engraft with the host myocardium, being well known that there is a direct correlation between cells' engraftment and LVEF improvement. According to Davis *et al.*, CSCd possess higher oxidative stress resistance in comparison with CSCs cultivated in single layer, and also enhanced expression of extracellular matrix molecules and adhesion molecules such as E-selectin, β1-laminin and α2-integrin. It is a proven fact that the molecular components of extracellular matrix and adhesion molecules have a key role in survival and grafting of cells, so that strategies to stimulate their expression could contribute to a superior grafting of a CSCd in the host heart, hence to a superior effect on the cardiac function [36].

An important aspect is the CSCs tropism for ischemic tissue. This was proven through identification of the transplanted cells in the necrotic area – even though those cells were injected at the border between the ischemic and healthy myocardium – and rapid disappearing of transplanted cells in the myocardium of healthy animals.

As aforementioned, the mechanism by which CSCs/CSCd contributes to the improvement of the cardiac function is not only direct, but also indirect. Currently, the dominant theory of stem cell effectiveness has moved toward the cytokine-paracrine hypothesis; the transplanted cells secret a large array of cytokines involved in cardioprotection and vasculogenesis [37]. Among the growth factors secreted by above-mentioned cells, there are vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1) and hepatocyte growth factor (HGF), their levels varying according to cultivation technique (cardiospheres or monolayer), as well as the time spent in culture. Activation of PI3K/AKT pathway by the stem cell derived factor (SCF) has a fundamental role in the migration of c-kit+ CSCs *in vivo* and *in vitro* [38], also improving the cells implantation [39, 40]; recently, Guo *et al.* attested that this effect is mediated, at least partially, by metalloproteinases (MMP) 2 and 9 [41].

CSC-based clinical trials

As expected, after preclinical studies, clinical trials were conducted in order to assess the efficacy of cardiac stem cell-based therapy for heart regeneration.

In case of SCIPIO (Stem Cell Infusion in Patients with Ischemic CardiOmyopathy) – phase one randomized trial – Bolli *et al.* reported improvement of LVEF by 12.3% one year after intracoronary autologous lin-/c-kit+ cells injection in patients with moderate to severe systolic dysfunction after myocardial infarction who underwent surgical revascularization. They also observed a decrease in infarct size, improvement in NYHA class and also quality of life in patients treated with SCs compared to control group [42]. However, in these circumstances, it is difficult to differentiate between the favorable outcome of myocardial revascularization and that of cell therapy.

Another phase one randomized trial – CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction) assessed the outcome of intracoronary CSCd (harvested by an endomyocardial biopsy) administration in different doses (12.5×10^6, 17.3×10^6, respectively 25×10^6) in patients with ischemic heart failure and LVEF between 25% and 40%. Magnetic resonance imaging (MRI) evaluation at 12 months after transplantation showed a decrease in scar size and myocardial viability

improvement in patients who received standard of care treatment plus CSCd *vs.* those who only received standard of care treatment, but with no effect on LVEF, left ventricular volumes, functional NYHA class or quality of life [43].

No adverse effects were reported in the follow-up period (12, respectively six months) in any of the mentioned trials.

The study conducted by Eduardo Marbán – "Allogeneic heart stem cells to achieve myocardial regeneration" (ALLSTAR) phase one trial, first of its kind, proposed to assess the safety and feasibility of allogenic CSCd intracoronary infusion in patients with recent (28–90 days) or chronic (91–365 days) myocardial infarction, with or without systolic dysfunction (mean LVEF 42% with variations between 26.7–55.1%) [44, 45]. Given the lack of serious adverse events, ALLSTAR II – phase II, randomized, double blind trial was initiated, still ongoing, with a design allowing the assessment of myocardial scar reduction by MRI.

Low rates in terms of migration, engraftment and survival of transplanted cells are one of the major limitations of cell therapy. Therefore, a number of strategies have been imagined and subsequently tested – preclinically [46, 47] as well as clinically – to stimulate the migration, engraftment and cell survival with more or less encouraging results. Effectiveness of administration of CSCs harvested by myocardial biopsy combined with hydrogels incorporating fibroblast growth factor in patients with ischemic heart failure and severe systolic dysfunction undergoing surgical revascularization is currently being tested in a small ongoing study in Japan: ALCADIA (AutoLogous Human CArdiac-Derived Stem Cell To Treat Ischemic cArdiomyopathy) phase one, non-randomized, without control group trial [48]. Of the six patients enrolled (five men and a woman), one was excluded from follow-up three weeks after surgery due to acute graft occlusion. Preliminary results are encouraging, the five remaining patients presenting – six months after combined therapy – improvement in LVEF (assessed by 2D echography and MRI), improvement in maximal oxygen consumption and reduction in infarct size. However, clinical use of growth factors/cytokines as adjuvants of cell therapy must be made with caution, given the potential risk of adverse effects [49].

Recent studies have brought into question quantitative but also qualitative changes of adult SC with old age, cardiovascular risk factors and associated comorbidities (diabetes, arterial hypertension or cardiovascular disease), decreasing the efficiency of cell therapy particularly in patients who need it most [50]. Even though most trials are focused on other types of SCs or progenitors cell (bone marrow SCs [51], adipose-derived stem cells [52–55], circulating endothelial progenitor cells [56–59]), there is direct evidence regarding CSCs damage in elderly individuals [60–63].

Conclusions and perspectives

Cardiac stem cell transplantation in ischemic disease proved to be both safe and feasible, but validation on larger number of patients is required. Also, accurate indications, optimal moment of cell administration and processing method remain to be determined, together with long-term effects. Future research efforts should concentrate on standardization of cell preparation and delivery procedures, as well as on developing new effective strategies to stimulate cell migration, engrafting and survival. Additional studies are necessary in order to determine the relationship between the active (contraction stimulation) and passive effect (remodeling inhibition) from quantitative and also temporal perspective. The last, but not the least, cell therapy should be personalized according to individual patient characteristics – including age and associated co-morbidities.

Conflict of interests

The authors confirm that there are no conflict of interests.

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