

Special Article

New Concepts in Cardiac Stem Cell Therapy

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*3rd Division of Cardiology, Medical University of Silesia, Katowice, Poland**Key words:***Stem cells, iPS, mesenchymal stromal cells, cardiac stem cells, genetic modification.**

In the last decade the concept of myocardial regeneration was rapidly translated from basic science and animal experiments to the application of bone marrow (BM)-derived stem and progenitor cells in multiple clinical trials. Meta-analyses and systematic reviews of these trials suggest that this form of cell therapy leads to modest improvement of left ventricular (LV) ejection fraction (LVEF) and reduces LV remodeling.^{1,2} The primary goal of all studies using stem and progenitor cells was to improve myocardial function and reduce remodeling by replacement of the fibrotic scar tissue with viable cardiac myocytes. Recently, the possibility of myocardial regeneration in humans has been challenged. Most probably, the benefits observed with BM cell therapy can be attributed to the paracrine and proangiogenic effects of injected cells.^{3,4} In the majority of clinical trials so far a heterogeneous population of non-selected BM-derived mononuclear cells was used, and currently there is no proof that any particular type of cells might be superior to others for myocardial repair in patients with acute myocardial infarction (MI) and ischemic cardiomyopathy.³

In parallel with the ongoing clinical trials using BM cells, investigating the optimal cell delivery route, dose and target population of patients, new concepts have emerged that aim to enhance the capability of BM cells to induce the functional recovery of the myocardium or to identify new, more potent cell populations. This

paper reviews the recent progress in experimental stem cell research that has currently been translated into clinical application.

Cardiac stem cells

Several investigators have demonstrated the presence of small clusters of Sca-1⁺, c-Kit⁺ or side population⁺ cells in the cardiac atria and apex. These cells were named cardiac stem cells (CSC) and they are most abundant during postnatal cardiac development in the first 2 weeks after birth. It has been postulated that asymmetric division of CSC leads to the formation of new cardiomyocytes.^{5,6} Progeny of CSC acquire a cardiomyocyte phenotype, so these cells seem to be optimal candidates for cardiac regeneration studies.⁷ CSC are self-renewing, but it is also likely that BM-derived stem cells, via their mobilization, can replace senescent and apoptotic CSC and participate in maintaining the CSC pool in the heart.^{8,9} In adulthood the cells are quiescent and reside within the niches. Following ischemic injury, CSC are activated by paracrine signals and start to divide. However, despite their proliferative potential, the extent of the myocardial necrosis following MI is too large to be compensated for by new cardiomyocytes formed by dividing CSC.^{9,10}

Experimental studies have demonstrated that CSC can be effectively isolated by percutaneous myocardial biopsy

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and expanded in culture to be used for myocardial repair.⁵ Application of CSC in experimental acute MI reduced LV remodeling, improved contractility and reduced the infarct size. Currently, there are several clinical trials that are evaluating the safety, feasibility and efficacy of CSC in patients with ischemic cardiomyopathy (www.clinicaltrials.gov; NCT00474461, NCT00981006). The cells can be either supplied via intracoronary infusion into the infarct zone or directly injected into dysfunctional but viable areas of the myocardium.¹¹

Genetically engineered bone marrow progenitor cells

As mentioned above, heterogeneous populations of BM cells have been used in the majority of the clinical studies investigating cellular therapy. The pool of mononuclear cells isolated from the BM by gradient centrifugation contains mostly committed cells, a small number of monopotent progenitor cells, and even fewer bona fide stem cells. The degree of improvement of myocardial contractility and perfusion observed in these trials is highly variable, and it is very likely that the effects are dependent on the functional quality and number of progenitor and stem cells that can be harvested from the individual patient.^{3,4,12,13} This in turn is dependent on age, comorbidities, medications and other factors. Numerous experiments have shown that in patients with diabetes there is a functional impairment of the BM-derived endothelial progenitor cells (EPC) and a lower number of circulating pluripotent cells in comparison to patients without diabetes. In addition, patients with ischemic cardiomyopathy have fewer EPC in the bone marrow, while the migratory capacity of these cells is significantly impaired.^{14,15} Other studies have demonstrated shortening of the telomeres in progenitor cells isolated from the BM and peripheral blood in patients with coronary heart disease.^{16,17} Therefore, if autologous cells are used for myocardial repair their functional status, which is dependent on the individual risk profile of the patients, might profoundly affect the outcome. It seems that a high proportion of patients enrolled in clinical trials with BM cells receive cellular product of suboptimal quality.^{14,15}

Recently, the view that the injected cells make a direct contribution to myocardial repair was challenged, and it seems more likely that the functional improvement observed in some trials can be attributed to the indirect paracrine effect of the cells. The possibility of genetic engineering of the cells has now

become accessible and could improve the functionality of the injected cells, including an increased release of vasoactive and protective substances. Another target for genetic manipulation would be the enhancement of signaling pathways, leading to improved cell survival, homing and engraftment.¹⁸

One of the factors critical for the mobilization of progenitor cells and the effects of cellular therapy is endothelial nitric oxide synthase (eNOS), which has also been shown to increase the migratory capacity of BM-derived cells. Overexpression or enhancement of eNOS improves the migration of stem cells induced by stromal cell-derived factor-1, which is a pivotal chemokine regulating cell mobilization, homing and engraftment. Such an approach increased the EPC-dependent angiogenesis in a hind-limb ischemia model.^{19,20} This approach will be evaluated in the enhanced angiogenic cell therapy – acute myocardial infarction (ENACT-AMI) trial, in which EPC engineered to overexpress eNOS are used. Peripheral blood-derived EPC will be separated by apheresis and transfected with human eNOS gene. In patients with acute MI, the use of modified EPC will be compared to unmodified EPC and placebo regarding their ability to increase LVEF after 6 months (www.clinicaltrials.gov; NCT00936819).

Allogeneic BM-derived cells

Because the functional impairment of BM-derived cells harvested from patients with chronic heart failure may limit the efficiency of such treatment, and since genetic engineering is not always possible, another way to overcome the obstacles would be to use allogeneic BM-cells isolated from healthy subjects. Mesenchymal stromal cells (MSC) are so far the best candidate for this approach, because they are immunoprivileged and escape rejection by the release of immunomodulatory factors and inhibition of T-cell proliferation. MSC make up a small proportion of BM cells (0.001-0.01% of nucleated cells in the bone marrow), but can be harvested from the BM and even more efficiently from the adipose tissue (ca. 1 million MSC per 100 cc of fat tissue). Importantly, the cells can be expanded *in vitro* and stored.^{21,22} This would allow the “off-shelf” treatment of patients with acute MI and severe left ventricular dysfunction, without the need to wait for the cell processing and expansion.²¹ There are already cellular products, such as Prochymal (Osiris Therapeutics, www.osiristx.com), that are being tested in patients with impaired LVEF

(www.clinicaltrials.gov; NCT00877903). The potential of MSC to undergo cardiomyocyte differentiation is still under dispute, but several experimental and clinical trials have shown their potential to improve LV function in ischemic cardiomyopathy. Because of their ability to undergo expansion, MSC can also be bioengineered to overexpress factors that increase their engraftment and differentiation.^{21,23} Apart from MSC, another population of potential interest for use in allogeneic transplantation is multipotent adult progenitor cells.²⁴

Very small embryonic-like stem cells

Adult bone marrow harbors various populations of cells that can potentially contribute to myocardial and endothelial repair, including a small subpopulation of non-hematopoietic cells that display the morphology and functional properties of embryonic pluripotent stem cells (PSC). In mice, using a FACS-based live cell sorting technique, a population of Sca-1⁺lin⁻CD45⁻CXCR4⁺ cells was isolated that showed enrichment in early cardiac and endothelial markers (GATA-4, Nkx2.5/Csx, VE-cadherin), and also several early embryonic markers (Oct-4, Nanog, SSEA-1) typical of embryonic PSC. Because of their small size (3-6 μm), presence of PSC markers, distinct morphology (open-type chromatin, large nucleus, narrow rim of cytoplasm with multiple mitochondria), and ability to differentiate into all three germ layers, these cells were named 'very small embryonic-like stem cells' (VSELs).²⁵⁻²⁷ We hypothesized that VSELs are derived from epiblast-stem cells and form a pool of quiescent PSC deposited in the BM, heart and other tissues during early organogenesis.²⁸ The presence of VSELs has been confirmed in several murine organs, including the heart and brain, as well as in umbilical cord blood and peripheral blood in adult humans.^{29,30} During experimental MI in mice and acute MI in patients there is a rapid mobilization of VSELs from the BM to the peripheral blood. Circulating VSELs are enriched in pluripotent markers as well as early cardiac and endothelial transcription factors.¹⁴

These findings suggest that the circulation of BM-derived VSELs may be an important mechanism of myocardial repair. Recently, we developed an ex vivo expansion and differentiation model that allows the generation of cardiomyocytes from BM-derived VSELs. In the first step, VSELs are plated in co-cultures with C2C12 myoblasts, where they form characteristic spheres resembling embryoid bodies. Subse-

quently, cells isolated from these spheres are plated on cardiac differentiation media to expand them into maturing cardiomyocytes (unpublished data).²⁵ The ability of BM-derived VSELs to undergo cardiomyocyte differentiation was the rationale for proof-of-concept experimental studies. In an experimental model of reperfused MI mice, direct intramyocardial injection of freshly isolated VSELs improved global and regional left ventricular contractility, and reduced myocardial hypertrophy. Interestingly, this effect was observed using only a small total number (10,000 cells) of injected VSELs. At the same time, a much higher number of hematopoietic cells (100,000 cells) was not effective.³¹ In addition, when the expanded VSELs underwent pre-differentiation for 5 days in the cardiogenic culture medium prior to the intramyocardial injection, they were markedly more effective in improving the LVEF and infarct wall thickening fraction than expanded but non-predifferentiated VSELs (Zuba-Surma EK, in press, *J Cell Mol Med*). The increased efficiency of pre-differentiated VSELs in terms of improved contractility and reduced remodeling were sustained over the 6-month follow up. Additionally, a limited number of VSELs-derived cardiomyocytes was documented (Zuba-Surma EK, *AHA 2008, Circulation 2008; 116: II204*). Clinical studies using autologous VSELs are needed to validate these promising experimental data.

Guided BM-derived mesenchymal cardiopoietic cells

Since pluri- and multipotent cells can differentiate into different lineages, and this population has no predilection for cardiac cells, the concept of guided cardiopoesis emerged, which is based on pretreatment of cells with factors that recapitulate the milieu characteristics of the early embryonic development of the heart and aims to restrict their differentiation potential to the cardiac lineage. The signals were identified using comparative genomic and proteomic analysis applied on the endodermal secretome, which in a paracrine manner guides the unipotent cardiomyocyte commitment of embryonic stem cells.^{32,33,34} This novel concept is currently being translated into clinical application and phase II and III clinical trials have been launched (C-Cure, safety, feasibility and efficacy of guided, autologous BM-derived mesenchymal cardiopoietic cells for the treatment of heart failure secondary to ischemic cardiomyopathy). The study will enroll 240 patients with New York Heart Association class II-III heart failure, reduced LV

ejection fraction (15-40%), and a history of previous myocardial infarction. Autologous BM-derived MSC will be isolated and processed with a cardiomyogenic “cocktail”, and subsequently injected into dysfunctional but viable myocardium using the NOGA system (www.clinicaltrials.gov; NCT00810238).

Induced pluripotent stem cells

Induced pluripotent stem cells (iPS) are the pluripotent cells generated by the transduction of adult somatic cells, such as fibroblasts, by overexpression of reprogramming factors, which are stem cell-associated genes (Oct3/4, Sox2, Klf4, c-Myc).^{35,36} Such epigenetic reprogramming leads to a change in the phenotype of the adult cells to resemble embryonic stem cells in terms of the presence of early embryonic markers (SSEA-1), teratoma formation in immunocompromised recipients, as well as their ability to generate cells from all three germ layers in chimeric animals after integration into an early-stage embryo. In contrast to embryonic stem cells, the use of iPS does not generate ethical controversies, while expansion in stem cell media can yield a sufficient number of cells that can subsequently be used for studies on cardiac differentiation. These cells are genetically identical to the donor cells.^{35,36}

The generation of murine iPS by Yamanaka et al was followed by reprogramming of human somatic cells using OCT4, NANOG, LIN28 and SOX2 genes.³⁷ The pluripotency of iPS was confirmed by demonstration of their capability for blastocyst complementation. Recently, murine iPS were generated using only 3 reprogramming factors (OCT3/4, Sox2, Klf4) to avoid the risk of tumorigenesis associated with c-Myc protooncogene.³⁸ The proof-of-concept studies showed that iPS can be efficiently differentiated into cardiac lineage cells that demonstrate the expression of early cardiac markers, followed by cardiac structural proteins, eventually leading to the formation of spontaneously contracting cardiomyocytes coupled by gap junctions. The pattern of the transmembrane calcium currents and action potential characteristics is similar to that of cardiomyocytes derived from embryonic stem cells; however, the electrophysiological properties of the iPS-derived cells differ in some aspects from those of embryonic stem cell cardiomyocytes, so the data need to be confirmed.^{38,39} In addition, murine iPS have been aggregated with preimplantation morula and produced chimeric mice that had functionally and anatomically normal hearts in which a significant

contribution from the iPS was demonstrated.⁴⁰ Importantly, iPS were used for cardiac regeneration in a murine model of MI. Intramyocardial injection of 2×10^5 iPS led to their stable engraftment within the myocardium of an immunocompetent recipient in up to 4 weeks. Delivery of iPS produced a marked improvement in contractility, while preventing LV remodeling and increased ventricular wall thickness. Histopathology data suggest that engrafted iPS contribute to myocardial and endothelial regeneration and reduce fibrosis.⁴⁰

The generation of iPS has inaugurated a new era in cardiac regeneration studies. However, unlike adult stem cells, iPS do show a potential to generate tumors, probably due to the insertional mutagenesis, so their use in humans cannot be envisioned in the near future. A possible way to overcome the risk is to use viral-free vectors and the proteins of reprogramming factors instead of DNA encoding.⁴¹ Also it is pivotal to investigate in detail whether iPS-derived cardiomyocytes represent cells that are capable of forming atrial, ventricular and pacemaker cells, as well as the conduction system. Furthermore, it needs to be established prior to any clinical use of iPS if this treatment is safe over long-term follow up.

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