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# Cardiac Cell Therapy: Boosting Mesenchymal Stem Cells Effects

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**Abstract** Acute myocardial infarction is a major problem of world public health and available treatments have limited efficacy. Cardiac cell therapy is a new therapeutic strategy focused on regeneration and repair of the injured cardiac muscle. Among different cell types used, mesenchymal stem cells (MSC) have been widely tested in preclinical studies and several clinical trials have evaluated their clinical efficacy in myocardial infarction. However, the beneficial effects of MSC in humans are limited due to poor engraftment and survival of these cells, therefore ways to overcome these obstacles should improve efficacy. Different strategies have been used, such as genetically modifying MSC, or preconditioning the cells with factors that potentiate their survival and therapeutic mechanisms. In this review we compile the most relevant approaches used to improve MSC therapeutic capacity and to understand the molecular mechanisms involved in MSC mediated cardiac repair.

**Keywords** Myocardial infarction · Cardiac regeneration · Cell therapy · Mesenchymal stem cells · Genetic engineering · Adult stem cells

## Introduction

Cardiovascular diseases are a major problem of world public health, being the leading cause of mortality and morbidity. Among them, heart failure triggered by acute myocardial infarction (AMI) is the main protagonist, causing the majority of deaths [1]. Although early reperfusion with fibrinolytic therapy or coronary angioplasty have reduced mortality [2], damage in the myocardial wall is irreversible and the available pharmacological and surgical treatments are limited to palliative effects. Thus, heart transplantation is the only effective approach in the later stages of AMI even though the low number of suitable donors restricts its application [3].

In AMI, severe ischemia induces apoptosis and necrosis of myocardial tissue [4] that is progressively replaced by fibrous tissue, due to the inability of the heart to regenerate itself [5]. This process leads to left ventricular remodelling characterized by left ventricular chamber dilatation, wall thinning and impairment of left ventricular function with a final stage of congestive heart failure and death [6].

Cardiac cell therapy is a new therapeutic strategy that could help to regenerate or to repair the injured cardiac muscle in order to prevent cardiac remodelling and heart failure. Many different stem/progenitor cells from a great variety of tissue sources have been used in experimental and/or clinical settings such as embryonic stem cells, MSC, hematopoietic stem/precursor cells (HSPC) neonatal or fetal cardiac stem cells, skeletal myoblasts and induced pluripotent stem cells (iPS) [7–10]. Several of these cell types have been employed in combination with different strategies to boost their positive effects like tissue engineering, genetic engineering or preconditioning with hypoxia or biological factors [11–13].

In the clinical setting, whole bone marrow mononuclear cells and skeletal myoblasts are the most frequently used cell types [14]. Overall, stem cells appear to be safe both in animal models and patients with minimal collateral effects [14–16]. However, it has been reported that skeletal myoblasts tend to induce arrhythmias due to lack of electrophysiologic integration with heart muscle and independent

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contractility [17–19]. Thus, the possibility that other types of cell precursors could induce arrhythmias with a low incidence cannot be discarded. Regarding the safety of MSC based therapies, there has been some concern about the tumorigenic potential of MSC [20], although it should be taken into account that human MSC possess a minimal risk of molecular transformation and preclinical and clinical studies have demonstrated their safety regard to cancer formation [21].

The efficacy of enriched stem cells populations in restoring cardiac function and promoting regenerative mechanisms such as revascularization and fibrosis reduction has also been demonstrated in clinical trials, but current results are modest and insufficient to repair injury of patients with AMI, due to several hurdles in heart stem cell therapy such as limited stem cell migration, survival, engraftment, proliferation and differentiation in the infarcted heart [14, 22].

This review focuses on MSC, one of the main types of stem cells used today in cardiac repair and extensively evaluated in diverse clinical settings [23–26], and explores the potential use of genetically manipulated MSC to increase their therapeutic potential.

## Mesenchymal Stem Cells: Main Features

MSC are multipotent cells that were identified by Friedenstein [27] and first isolated from bone marrow stroma by Pittenger and coworkers in 1999 [28]. These cells are able to differentiate into adipocytes, chondrocytes and osteoblasts in vitro [29–31] and to engraft and differentiate into multiple tissues following in utero transplantation [32]. Although abundance of MSC in fresh bone marrow is low (0.01–0.0001%) [33] they can be easily expanded due to their ability to adhere to plastic surfaces and their proliferative potential [34].

MSC have been isolated from adult pheripheral blood [35], adipose tissue [36], skin tissue [37], dental pulp [38], liver [39], synovial membrane [40], skeletal muscle [41], lung [42], umbilical cord blood [43], amniotic fluid [44] and placenta [45], amongst others [46]. MSC isolated from different tissues share common antigenic markers, namely CD13, CD29, CD31, CD44, CD54, CD63, CD73, CD90, CD105, CD106, CD140b and CD166 and are negative for the antigenic markers on hematopoietic stem cells (CD34, CD45, CD14 and CD133) [47]. However, studies of microarrays show differences in gene expression and multilineage differentiation depending on their source of origin [48].

MSC can be expanded for up to 29 population doublings before entering into senescence, although they tend to produce better outcomes when they are isolated from young donors [49]. Indeed, Asumda and colleagues found lower expression of Oct 4 in MSC isolated from young rats (4 month old) than from older animals (15 months) [50]. The authors failed to detect Sox2 and Nanog in "old" BM-MSC and were able to induce cell differentiation after 21 days culture in adipogenic, osteogenic and chondrogenic differentiation media. Accordingly, the telomerase activity and secretion of paracrine factors was higher in young MSC.

MSC are an attractive cellular type for cardiac therapy because they are relatively easy to obtain from different tissue sources like bone marrow or adipose tissue.

These cells have been reported to be immunoprivileged due to lack of or low levels of surface expression of MHC class I and MHC class II molecules [51] which enables them to evade detection by T cells in an allogeneic setting [52]. Furthermore, MSC have demonstrated immunosuppressive properties through modulation of cellular and innate immune pathways [53, 54]. However, it should be taken into account that some authors have reported immune rejection of allogeneic MSC [55, 56]. These features, together with the paracrine effect through secretion of angiogenic, anti-apoptotic and anti-fibrotic factors, are the most likely therapeutic mechanisms by which MSC are able to attenuate the pathological effects of cardiac remodelling in AMI, increasing angiogenesis, reducing ventricular dilatation and improving global cardiac function [57–59].

## MSC Direct Effects in Cardiac Therapy: Migration, Engraftment and Differentiation

During the first 7 days after myocardial infarction a complex and acute inflammatory process is observed [60] that provokes the release of a great variety of chemokines, growth and inflammatory factors by the ischemic tissue. This injury response attracts and induces recruitment of different types of leukocytes that promote healing [61, 62]. Inflammatory factors released shortly after the infarction include IL-8, IL-10, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein 1α and 1β (MIP-1α and MIP-1β), HGF and SDF-1 [60, 63, 64]. Several of these up-regulated biological factors post-AMI have also been reported to be involved in MSC migration into infarcted tissue like SDF-1 and HGF [65, 66]. In this context, MSC express CXCR4 and c-Met which are receptors of SDF-1 and HGF respectively. Treatment of MSC with proinflammatory cytokines increase adhesion and susceptibility of these cells to migrate in respond to trophic factors [67, 68], indicating the ability of MSC to exert an adaptive response to inflammatory signals. Concomitantly, once MSC migration process starts they need to adhere and go through endothelium in order to reach myocardial infarction. Several adhesion molecules and integrins have been identified in MSC membrane surface such as vascular cell adhesion molecule-1 (VCAM-1), very late antigen-4 (VLA-4), intercellular adhesion molecule-1/3 (ICAM-1 and

ICAM-3), β1 integrins, activated leukocyte-cell adhesion molecule (ALCAM) and CD44 [65, 69, 70]. Moreover, MSC secrete matrix metalloproteinases such as MMP-2 which facilitate invasion into infarcted heart [71]. In this context, VCAM-1, VLA-4, β1 integrins and matrix metalloproteinase-2 (MMP-2) secretion have been reported to be key players involved in MSC adhesion and/or transendothelial migration to infarcted tissue [71, 72].

Engraftment of MSC has been specifically addressed in several studies. In general, MSC are capable of engraftment in the host myocardium, but the percentage of retained cells is quite low. The best engraftment results using MSC from humans in transplantation experiments have been achieved using immunodeficient animals to prevent cell rejection [73]. Despite the relative immunoprivileged nature of MSC, no engraftment was found 7 days after xenogenic transplantation into immunocompetent rats [74, 75]. To our knowledge, the longest engraftment of MSC in large animal models was reported by Quevedo and colleagues [76]. Using a swine allogenic model, $2\times10^8$ male MSC were injected in chronically infarcted female swine (12 weeks after MI) and were detected 12 weeks post-transplantation by colocalization with Y-chromosome fluorescence in situ hybridization. In these conditions, only a small percentage of MSC were able to engraft and differentiate into cardiomyocytes at this time point (less than 600 cells per $10^6$ cardiomyocytes). Interestingly, the same research group reported that transplantation of these cells was able to stimulate endogenous cardiomyocyte cell cycling and amplify resident c-kit+cardiac resident stem cells 2 weeks after injection [77]. Muller-Ehmsen and colleagues evaluated the mid-term persistence of bone marrow mononuclear cells (BMNC) and MSC in rat models of acute and chronic myocardial infarction [78]. BMNC or MSC were injected into myocardium immediately or 7 days after MI. The study showed that after 6 weeks post-implantation the percentage of engrafted cells was around 0.3–3.5% independently of cell type and application time. Overall, the engraftment percentage of administered cells rapidly decreased due to poor mid-term persistence.

Engraftment efficiency is closely correlated with the mode of administration of cells. Both intracoronary and endocardial MSC injections showed an increased engraftment within infarcted tissue when compared with intravenous infusion since the later produce higher mortality and loss of transplanted cells due to entrapment through the pulmonary circulation in lung, liver and spleen. Intracoronary injections produced a decrease in blood flow whereas endocardial injections resulted in similar engraftment but reduced collateral effects [79]. Thus, cell transplantation is still an unsolved problem and methods able to increase the retention of cells in the heart will undoubtedly increase the efficacy of cell therapy. In this context, the use of scaffolds to deliver cells, loaded or not with growth factors, will also help to improve the long term viability of stem cells in solid organs [80–83]

MSC are able to initiate differentiation in vivo into muscle or endothelium. Although few MSC engrafted in injured heart frequently express some muscle, cardiac and/or endothelial marker proteins such as smooth muscle alpha-actin, desmin, β-myosin heavy chain, α-actinin and cardiac troponin T [73, 84, 85] currently, general consensus indicates that this process is extremely rare and may be product of differentiation or cell fusion [86, 87]. However, whatever mechanism, this minor differentiation process is not sufficient to explain the restorative mechanisms observed after MSC cell therapy in AMI [10, 86, 88]. Besides, MSC beneficial effects appear shortly after cell transplantation (around 3 days), which would give an insufficient time for MSC to differentiate into cardiac lineages [86, 89].

## MSC Indirect Effects in Cardiac Therapy: Paracrine Factors

MSC are able to release a great variety of cytoprotective cytokines and growth factors which are implicated in protecting injured tissue from apoptosis, promoting angiogenesis, reducing infarct scar and preventing tissue remodelling [57, 90]. The following molecules can be found among the most relevant cytokines and growth factors secreted by MSC: Vascular endothelial growth factor (VEGF), beta-fibroblast growth factor (βFGF), insulin-like growth factor (IGF-1), stromal cell-derived factor-1 (SDF1), transforming growth factor beta (TGFβ), and IL-6 interleukins, hepatocyte growth factor (HGF), angiopoietin-1 (Ang-1) and platelet-derived growth factor (PDGF) [91], monocyte/macrophage colony stimulating factor (M-CSF) and granulocyte colony stimulating factor (G-CSF) [92] (Table 1). At present, it is firmly suggested that the main mechanism responsible for cardiac repair in MSC is the secretion of paracrine factors rather than MSC differentiation into cardiomyocytes [89, 93, 94]. Indeed, several studies have reported that culture of endothelial cells with conditioned medium released by MSC resulted in improved angiogenesis, migration and survival in vitro and that intramyocardial injection of MSC conditioned medium in animals with AMI resulted in functional improvement, increased capillary density and reduction of infarct size [95–98]. In this context, there is intense research to define the best combination of factors and also the adequate way of administration of cells to the infarct with the use of, for example, polymeric carriers that allowed the delivery at the appropriate dose. These composite scaffolds, encapsulating cells or factors, could mimic the effects of intramyocardial cell therapy whilst at the same time reducing the complexity and cost of therapy in humans [80].

**Table 1** Cytokines and growth factors secreted by MSC

| Cytokine/Growth factor | General role |
|---|---|
| VEGF | Stimulation and regulation of angiogenesis and vasculogenesis [133] |
| bFGF | Stimulation of the proliferation of multiple cell types [134, 135] |
| IGF-1 | Stimulation of the proliferation and growth of multiple cell types [136] |
| SDF1 | Chemotactic factor for monocytes, lymphocytes, megakaryocytes and hematopoietic cells [137] |
| TGFβ | Regulation of cell proliferation and differentiation [138, 139] |
| IL-6 | Mediator of inflammation and acute phase reaction [140] |
| HGF | Stimulation of the proliferation and motility of epithelial and endothelial cells [141] |
| PDGF | Regulation of cell proliferation and angiogenesis [142] |
| G-CSF | Stimulation of neutrophil proliferation and differentiation [92] |
| M-CSF | Stimulation of monocyte proliferation and differentiation [92] |
| Ang-1 | Stimulation and regulation of angiogenesis and vasculogenesis [143] |

*VEGF* vascular endothelial growth factor, *bFGF* basic fibroblast growth factor, *IGF*-1 insulin growth factor 1, *SDF* serum derived factor, *TGF* tumor growth factor, *IL*-6 interleukin 6, *HGF* hepatocyte growth factor, *PDGF* platelet derived growth factor, *G-CSF* Granulocyte colony-stimulating factor, *M-CSF* Macrophage colony-stimulating factor, *Ang*-1 angiopoietin 1

## Boosting Stem Cell Effects

In spite of MSC ability to trigger therapeutic biological processes that contribute to cardiac repair, the use of these cells produce only modest improvements in cardiac function and the beneficial effects in humans with myocardial infarction are far from clinical implementation. As a result, many different strategies are being developed in order to boost these cell effects such as genetic engineering, tissue engineering and pre-treatments with biological factors. Tables 2, 3 and 4 summarize studies performed using viral vectors to overexpress transcription factors, cytokines or growth factors in MSC prior to administration. Although almost all of these molecules show pleiotropic effects, they have been classified by their main mechanism of action.

## Strategies to Improve MSC Engraftmet and Differentiation

After MI, chemotactic factors are upregulated in injured tissues. Homing and engraftment of MSC in infarcted myocardium is associated with various chemokine/chemokine receptor axes including SDF-1α (CXCL12) and its receptor CXCR4, HGF and its receptor cMet and CXCL1 and its receptor CCR1. In order to explore their role in cell engraftment, several groups have genetically modified MSC to potentiate these mechanisms and to test their therapeutical potential in vitro and in vivo. Approaches to improve cell engraftment have been mostly conducted with murine (mice or rats) syngeneic models. Overexpression of CCR1 but not CXCR2 led to improved cardiac function and vascular density, reduced infarct size, and increased release of paracrine factors in vivo, in comparison with MSC treated animals [99].

Implications of SDF-CXCR4 interactions in MSC induced cardiac repair have been extensively studied either by overexpressing the ligands or the receptors in MSC prior to transplantation [99–102]. Increased engraftment of MSC overexpressing CXCR4 (CXCR4-MSC) versus MSC was demonstrated by Y-chromosome positive cell staining and by localization of GFP expressing cells at the border zone of the infarct. CXCR4-MSC also increased paracrine activity of infused cells with an upregulation of matrix metalloproteinases (MMPs) in CXCR4-MSC transplanted hearts [100]. When comparing studies from different groups that used similar animal models, higher doses were required to obtain similar therapeutic benefits when using the intravenous infusion (i.v) than intramyocardial injection (IM) (Table 2). Two different studies demonstrated that infusion of SDF-1 overexpressing MSC significantly improved stem cell engraftment (up to 5 fold relative to non-modified MSC), as well as decreasing the number of TUNEL positive cardiac myocyte nuclei and improving cardiac function [101, 102]. In one of the studies, MSC were labelled with BrdU prior to injection [101]. No evidence of cardiac regeneration by the infused MSC being derived from replicating cells was observed, and the authors demonstrated that the beneficial effects of stem cell transplantation were associated to cardiac preservation rather than to cardiac regeneration.

In another study, monitoring of MSC engraftment by luminescence in vivo showed that overexpression of HGF and VEGF prolonged MSC short term engraftment (2–6 days) [103]. However, the authors failed to detect MSC, overexpressing or not these growth factors, at 10 days post-transplantation, indicating that the incidence of these genetic modifications only improve short term engraftment. Nevertheless, in most cases, this presence is sufficient to induce long lasting therapeutical benefits due possibly to paracrine mechanisms and induction of stem cell homing [101, 102, 104].

Regarding the studies directed to potentiate the mechanisms implicated in MSC differentiation, some studies are based on genetic modifications with cardiac transcription factor genes or kinases that regulated various intracellular functions [105–107]. Myocardin is a cardiomyogenic transcription factor that regulates the expression of many

**Table 2** Improvement of MSC engraftment and tissue repair in animal models of experimental infarction

| Genetic modification | Cell dose and labeling system | Animal model | Route of delivery and trasplantation schedule | Proposed mechanism of action and engraftment occurrence | Cardiac parameters and registration time | In vivo results | Ref. |
|---|---|---|---|---|---|---|---|
| CCR1 overexpression | Mixture of $0.75\times10^5$ LacZ-MSC and $3\times10^5$ GFP-labeled mice MSC | Mouse | IM injection 1 h after LAD ligation | ↑ MSC migration and survival through CXCL1/CCR1 axis. Engraftment after three days post-t | **ECHO**, ΔFS (%): 9. 28 days after AMI | ↓ CM apoptosis ↑ vascular density ↓ infarct size | [99] |
| CXCR4 overexpression | $2\times10^6$ male rat GFP-labeled MSC | Female rat | i.v infusion 3 d after LAD ligation | Facilitation of engraftment by up-regulation of matrix metalloproteinases | **ECHO**, ΔEF (%): 12.1. 28 days after AMI | ↑ mobilization and engraftment into infarcted area ↑ vascular density ↓ infarct size | [100] |
| CXCR4 overexpression | $2.5\times10^6$ DiI- labeled rat MSC | Rat | i.v infusion 24 h after I/R | ↑ MSC homing through SDF-1/CXCR4 axis | **ECHO and HM** ΔEF (%): 15 ΔFS (%): 10. 31 days after AMI | Cardiac function improvement. ↑ mobilization and engraftment into infarcted area | [144] |
| Myocardin overexpression | $2\times10^5$ human MSC | Mouse | IM injection shortly after LAD ligation | Stimulation of cardiomyogenic phenotype | **MRI and HM** ΔEF (%): 2. 14 days after AMI | ↑ engraftment and induction of cardiomycyte-like phenotype | [108] |
| SDF-1 overexpression | $2\times10^6$ GFP-labeled rat MSC | Rat | i.v infusion 24 h after LAD ligation | ↑ Homing, survival and growth of CXCR4 expresing MSC | **ECHO**, ΔFS (%): 17.3. 35 days after AMI | ↑ CM survival ↑ vascular density | [101] |
| SDF-1 overexpression | $0.5\times10^6$ GFP-labeled rat MSC | Rat | IM injection 5 d after LAD ligation | ↑ MSC tolerance to hypoxia partly mediated by antifibrotic factors such as HGF | **HM** 33 days after AMI | ↑ MSC survival ↑ vascular density ↓ infarct size | [102] |
| sFPR2 overexpression | $2.5\times10^5$ GFP-labeled mouse MSC | Mouse | IM injection shortly after LAD ligation | ↑ MSC engraftment and proliferation through suppression of BMP and Wnt signaling | **ECHO,** ΔEF (%): 6.24. ΔFS (%): 13.8. 30 days 1after AMI | ↑ engraftment ↑ vascular density ↓ infarct size | [109, 111] |

*DiI* 1,1′-dioctadecyl-3,3,3′3′-testramethylindocarbocyanine perchlorate. *EF* ejection fraction. *FS* shortening fraction. *AMI* acute myocardial infarction. *ECHO* echocardiography. *HM* hemodynamic measurements. *IM* Intramyocardial. *i.v* intravenous. *IC* intracoronary. *LAD* left anterior descendent artery. *I/R* ischemia-reperfusion. The mean percentage difference (Δ%) values of cardiac function parameters between genetically modified MSC and control MSC are indicated

**Table 3** Improvement of MSC survival and tissue repair in animal models of experimental infarction

| Genetic modification | Cell dose | Animal model | Route of delivery and trasplantation schedule | Proposed mechanism of action | Cardiac parameters nd registration time | In vivo results | Ref. |
|---|---|---|---|---|---|---|---|
| Akt overexpression | $1\times10^7$ GFP- swine MSC | Swine | IC injection 3 d after LAD ligation | ↑ MSC resistance to apoptosis and survival through ERK and VEGF ↓ ROS levels | **SPECT,** ΔEF (%): 8.5. 31 days after AMI | ↑ MSC survival ↓ infarct size | [120] |
| Akt overexpression | $1\times10^7$ swine MSC | Swine | IC injection | ↑ MSC resistance to apoptosis ↑ expression of VEGF | **ECHO and MRI** ΔEF (%): 7.66. 56 days after AMI | ↓ infarct size, ↑ vascular density and ↑ MSC survival | [121] |
| Akt overexpression | $5\times10^5$ GFP-mouse MSC | Mouse | IM injection 1 h after LAD ligation | Cytokines and growth factors modulation by Akt | **ECHO,** ΔEF (%): Q.D. 28 days after AMI | ↑ transient engraftment | [86] |
| Akt overexpression | $2.5\times10^5$ and $5\times10^6$ LacZ/GFP-rat MSC | Rat | IM injection 1 h after LAD ligation | ↑ MSC viability through survival signals | **HM** 19 days after AMI | ↓ intramyocardial inflammation, collagen deposition and cardiac myocyte hypertrophy | [112] |
| Ang-1 and Akt overexpression | $3\times10^6$ Lac Z-rat MSC | Rat | IM injection shortly after LAD | ↑ Angiogenesis and MSC graft survival by Ang1 and Akt signalling pathways | **ECHO,** ΔEF (%): 12.55. ΔFS (%): 8.26. 28 days after AMI | ↓ infarct size ↑ vascular density and MSC survival | [113] |
| Ang-1 and Akt overexpression | $3\times10^6$ Male rat MSC | Female Rat | IM injection shortly after LAD | ↑ Angiogenesis, MSC graft survival and proliferation | **ECHO,** ΔEF (%): 15.83. ΔFS (%): 9.49. 84 days after AMI | ↑ vascular density | [114] |
| Bcl-2 overexpression | $6\times10^6$ GFP-rat MSC | Rat | IM injection shortly after LAD | ↓ MSC apoptosis and ↑ resistance to ischemia | **HM,** ΔEF (%):4.16 42 days after AMI | ↑ vascular density | [124] |
| Connexin 43 overexpression | $5\times10^5$ rat MSC | Rat | IM injection | ↑ Bcl-2 and phosphorylated Akt ↑ Resistance to hypoxic injury | **ECHO,**ΔEF (%): 5.4. ΔFS (%): 5. 21 days after AMI | ↓ infarct size ↑ MSC survival | [125] |
| FGF-2 overexpression | $5\times10^5$ GFP-rat MSC | Rat | IM injection shortly after cryoinjury | ↑ MSC survival and CM differentiation | Without cardiac function studies | ↑ MSC survival and angiogenesis | [145] |
| GATA-4 overexpression | $1.5\times10^6$ GFP-rat MSC | Rat | IM injection following LAD ligation | MSC resistance to oxidative stress | **ECHO,** ΔEF, ΔFS: Q.D. 28 days after AMI | ↑ vascular density and MSC survival, ↓ infarct size | [146] |
| GSK-3β overexpression | $1,5\times10^5$ Lac Z- mouse MSC | Mouse | IM injection following LAD ligation | ↑ MSC survival, CM differentiation and angiogenesis through VEGF-A mechanisms | **ECHO and HM** ΔFS (%): Q.D. 84 days after AMI | ↑ MSC survival ↑ vascular density | [107] |
| HO-1 overexpression | $1\times10^6$ Lac Z-mouse MSC | Mouse | IM injection 1 h after LAD ligation | Anti-apoptotic and anti-oxidative effects by HO-1 | **HM** 14 days after AMI | ↑ MSC survival | [115] |
| HO-1 overexpression | $5\times10^6$ GFP-rat MSC | Rat | IM injection 1 h after LAD ligation | Anti-apoptotic and anti-oxidative effects by HO-1 through PI3K/Akt pathway | **ECHO** ΔEF (%): 4.5. ΔFS 2.9. 28 days after AMI | ↑ MSC survival, VEGF production ↑ vascular density | [116] |
| HO-1 overexpression | $1\times10^6$ rat MSC | Rat | IM injection 1 h after LAD ligation | ↑ Angiogenesis through enhanced VEGF and FGF2 | **HM** 28 days after AMI | ↓ fibrotic area and apoptosis ↑ vascular density | [117] |
| HO-1 overexpression | $1\times10^7$ Lac Z-swine MSC | Swine | IC injection 1 h after LAD ligation | ↑ Angiogenesis through enhanced FGF2 | **MRI, ECHO** ΔEF (%): 4.37 28 days after AMI | ↑ vascular density | [118] |
| HO-1 overexpression | $1\times10^6$ rat MSC | Rat | IM injection 1 h after LAD ligation | Reversion of extracellular remodeling | **ECHO,** ΔFS (%): Q.D. 7 days after AMI | ↑ vascular density | [119] |
| Hsp20 overexpression | $1\times10^6$ GFP-rat MSC | Rat | IM injection 10 min after LAD ligation | ↑ Akt activation and ↑ secretion of growth factors (VEGF, FGF-2 and IFG-1) | **ECHO,** ΔEF (%): 12. ΔFS (%): 12. 28 days after AMI | ↑ MSC survival, vascular density and ↓ fibrosis | [122] |
| ILK overexpression | $1\times10^6$ rat MSC | Rat | IM injection | ↑ MSC survival, adhesion and ERK1/2 and Akt activation | Without cardiac function studies | ↓ infarct size and apoptosis ↑ vascular density | [131] |
| | $1\times10^6$ GFP-rat MSC | Rat | | | | | [123] |

**Table 3** (continued)

| Genetic modification | Cell dose | Animal model | Route of delivery and trasplantation schedule | Proposed mechanism of action | Cardiac parameters nd registration time | In vivo results | Ref. |
|---|---|---|---|---|---|---|---|
| PI3K-C2α overexpression | | | IM injection shortly after LAD ligation | ↑ MSC survival through production of pro-survival factors | **HM,** ΔEF (%): 11.5. 21 days after AMI | ↓ fibrotic area, infarct size and apoptosis ↑ Vascular density | |
| Survivin overexpression | $2\times10^6$ GFP-rat MSC | Rat | IM injection 1 h after LAD ligation | ↑ MSC survival through anti-apoptotic action of survivin | **ECHO,** ΔEF (%): 25. 28 days after AMI | ↑ MSC survival ↑vascular density ↓ infarct size | [147] |
| tTG overexpression | $1\times10^6$ DAPI-rat MSC | Rat | IM injection shortly after LAD ligation | ↑ MSC integrin-mediated adhesion 3 d post-transplantation | **ECHO,** ΔEF (%): 22.1. ΔFS (%): 13.62. 21 days after AMI | ↑ MSC survival and adhesion | [132] |

*CM* cardiomyocyte. *EF* ejection fraction. *FS* shortening fraction. *AMI* acute myocardial infarction. *ECHO* echocardiography. *MRI* magnetic resonance imaging. *HM* hemodynamic measurements. *SPECT* single-photon emission computed tomography. *CT* computed tomography. *IM* intramyocardial. *IV* intravenous. *IC* intracoronary. *Q.D.* qualitative data. The mean percentage difference (Δ%) values of cardiac function parameters between genetically modified MSC and control MSC are indicated

cardiac and smooth muscle cell genes. Myocardin expression in MSC increased differentiation of transplanted cells [108], although full differentiation was not achieved and the vast majority of engrafted cells were only positive for one of the cardiac markers analysed (i.e. cardiac troponin T, atrial natriuretic peptide, and myosin heavy chain, among others). However, although Myocardin-MSC showed improved functional cardiac parameters in comparison with MSC, the differences were low with only an increase in ejection fraction of 2% relative to MSC treatment, 14 days after cell transplantation. In contrast, genetic modifications leading to improved MSC self-renewal and survival seem to induce higher levels of cardiac function recovery. For instance, overexpression of MSC with sFPR2, an inhibitor of the Wnt pathway that has been associated with increased healing capacity, together with MSC survival and proliferation [109, 110], the same factor was also able to increase the ejection fraction in 6.24% using the same animal model and the same cell dose as the Myocardin study, but 30 days after cell transplantation [111], indicating that beneficial effects induced by MSC are more influenced by the ability of MSC to survive in the in the host than by the degree of MSC differentiation.

## Strategies to Improve MSC Survival and Proliferation

Strategies conducted to improve MSC survival have been often developed using murine animal models [86, 112–119] although some of the experiments have been performed in swine [120, 121] (Table 3).

The first genetic modification approach directed to improve MSC survival was reported by the group of Dr. V.J. Dzau [112]. Transplantation of Akt overexpressing MSC in infarcted rats significantly improved cardiac function and reduced infarct size in comparison with MSC treated animals in a dose dependent manner. Lim and colleagues [120] injected MSC intracoronary in a swine model of ischemia-reperfusion. Cells were injected 3 days after balloon occlusion to avoid interference with the inflammation cascade triggered after MI. In these conditions, Akt overexpressing cells significantly preserved cardiac function. In vitro, the authors demonstrated the ability of Akt-MSC to reduce intracellular ROS levels induced by $H_2O_2$ treatment. In these modified cells, AKT and ERK were found to remain in phosphorylated form longer than in MSC. Akt overexpression induced transient beneficial effects related to paracrine mechanisms. Since angiogenesis is a major mechanism of repair and to obtain long lasting therapeutical benefits, further studies were conducted by co-expression of Akt and angiopoietin (Ang-1) in MSC. After 3 months following Akt-Ang-1-MSC transplantation, authors detected improved cell engraftment and increase in blood vessel maturation index [113, 114]. Other authors

**Table 4** Improvements of MSC paracrine effects/functionality and tissue repair in animal models of experimental infarction

| Genetic modification | Cell dose | Animal model | Route of delivery and trasplantation schedule | Proposed mechanism of action | Cardiac parameters and registration time | In vivo results | Ref. |
|---|---|---|---|---|---|---|---|
| Adrenomedulin overexpression | $5\times10^6$ Luc-rat MSC | Rat | IM injection following LAD ligation | Adrenomedullin anti-apoptotic and angiogenic effects | **ECHO and HM,** ΔFS (%): 6. 28 days after AMI | ↓ infarct size and apoptosis ↑ vascular density | [148] |
| Ang-1 overexpression | $5\times10^6$ GFP-rat MSC | Rat | IM injection following LAD ligation | ↑ Angiogenesis by Ang-1 | **ECHO,** ΔFS (%): -0.4. 28 days after AMI | ↓ infarct size ↑ vascular density | [149] |
| Angiogenin overexpression | $6\times10^8$ DiI labeled-swine MSC | Swine | IM injection 4 weeks after occlusion | ↑ Angiogenesis by angiogenin | **ECHO and MRI** ΔEF (%): Q.D. 56 days after AMI | ↓ infarct size ↑ vascular density | [150] |
| Angiogenin overexpression | $5\times10^6$ DiI labeled rat MSC | Rat | IM injection 1 d after LAD | ↑ Angiogenesis and MSC survival by angiogenin | **ECHO,** ΔEF and ΔFS: Q.D. 42 days after AMI | ↓ infarct size ↑ MSC survival ↑ vascular density | [105] |
| HGF overexpression | $5\times10^6$ rat MSC | Rat | IM injection | ↑ Angiogenesis by HGF | **HM** 28 days after AMI | ↓ infart size ↑ vascular density | [103] |
| HGF overexpression | $5\times10^6$ rat MSC | Rat | IM injection 4w after LAD | ↑ Angiogenesis and ↓ apoptosis partly mediated by upregulation of calcineurin, Akt and Bcl2 | **ECHO and HM** ΔFS (%): 5.8. 56 days after AMI | ↑ vascular density | [126] |
| HGF/VEGF overexpression | $0.5\times10^6$ Luc-mouse MSC | Mouse | IM injection following LAD ligation | ↑ Tolerance of cardiomyocytes to ischemia, ↑ survival by ↓ apoptosis and ↑ Akt activation | **CT,** ΔEF: 10% 180 days after AMI | ↓ scar size ↑ vascular density | [127] |
| IGF-1 overexpression | $1.5\times10^6$ rat MSC | Rat | IM injection following LAD ligation | ↑ MSC survival and Stem cell mobilization via SDF-1 signaling | **ECHO,** ΔEF (%): 10.1. ΔFS (%): 6.14. 28 days after AMI | ↑ vascular density and MSC survival ↓ infart size | [104] |
| IL-18BP overexpression | $1\times10^6$ mouse MSC | Rat | IM injection | ↑ VEGF production and MSC survival ↓ IL-6 levels | **ECHO,** ΔEF (%): 10.4. ΔFS (%): 7.7. 28 days after AMI | ↓ infarct size | [151] |
| MiR-126 overexpression | $5\times10^6$ GFP-mouse MSC | Mouse | IM injection 2w after LAD ligation | ↑ Angiogenesis by stimulation of AKT/ERK-related pathway | **ECHO,** ΔEF (%): 13. ΔFS (%): 14. 6 weeks after transplantation | ↑ vascular density | [130] |
| Notch1 intracellular domain overexpression | $5\times10^5$ GFP-mouse MSC | Mouse | IM injection following LAD ligation | ↑ Neovascularization | **ECHO,** ΔEF (%): 8.8. ΔFS (%): 5. 28 days after AMI | ↓ infarct size | [152] |
| PGIS overexpression | $5\times10^4$ mouse MSC | Mouse | IM injection | ↓ Apoptosis and inflammation ↑ early neo-vascularization | **ECHO,** ΔEF (%): 5.2. ΔFS (%): 3.6. 14 days after AMI | ↓ myocardial fibrosis ↑ levels of VEGF | [153] |
| TNFR overexpression | $1\times10^7$ rat MSC | Rat | IM injection | Attenuated protein production and gene expression of inflammatory cytokines TNF-α, IL-1b and IL-6 in myocardial infarction | **ECHO,** ΔEF (%): 14.8. ΔFS (%): 6.8. 14 days after AMI | ↓ Cardiomyocytes apoptosis | [154] |
| TNFR overexpression | $1\times10^7$ rat MSC | Rat | IM injection | ↑ MSC survival by reduced levels of TNF-α in serum and cardiac tissue | **ECHO and HM** ΔEF (%): 14.8. 14 days after AMI | ↑ MSC survival | [155] |
| VEGF overexpression | $3\times10^6$ rat MSC | Rat | IM injection | ↑ Angiogenesis by VEGF | **HM** 42 days after AMI | ↓ infarct size ↑ vascular density | [128] |
| VEGF overexpression | $8\times10^6$ DiI-rat MSC | Rat | IM injection following LAD ligation | ↑ Angiogenesis by VEGF | **ECHO and HM,** ΔEF: Q.D. 1 month after AMI | ↓ infarct size ↑ vascular density | [106] |

**Table 4** (continued)

| Genetic modification | Cell dose | Animal model | Route of delivery and trasplantation schedule | Proposed mechanism of action | Cardiac parameters and registration time | In vivo results | Ref. |
|---|---|---|---|---|---|---|---|
| VEGF overexpression | $5\times10^6$ Lac Z-human MSC | Rat | IM injection 1w after LAD ligation | ↑ Angiogenesis SDF-1α/ CXCR4-mediated recruitment of cardiac stem cells | **HM** 28 days after AMI | ↑ vascular density<br>↓ infarct size | [95] |
| VEGF overexpression under hypoxic conditions | $2\times10^6$ rat MSC | Rat | IM injection | ↑ Angiogenesis by VEGF<br>↑ MSC homing in infarcted myocardium | **ECHO,** ΔEF (%): 13.7. ΔFS (%): 4.6. 14 days after AMI | ↓ infarct size and fibrotic area ↓ MSC apoptosis<br>↑ vascular density | [129] |

*EF* ejection fraction. *FS* shortening fraction. *ECHO* echocardiography. *MRI* magnetic resonance imaging. *HM* hemodynamic measurements. *AMI* acute myocardial infarction. *IM* Intramyocardial. *Luc* Luciferease. *Q.D.* qualitative data. The mean percentage difference (Δ%) values of cardiac function parameters between genetically modified MSC and control MSC are indicated

overexpressed PI3K-C2α or heat shock protein 20 (Hsp 20) in MSC to potentiate the Akt survival pathway, and obtained similar results in terms of improvement of cardiac function and cell engraftment [122, 123].

The second mostly studied strategy to improve MSC therapeutic potential has been the overexpression of heme oxygenase 1(HO-1), an anti-oxidant and anti- inflammatory protein that catalyzes the enzymatic degradation of heme to carbon monoxide, biliverdin and iron. HO-1 is sensitive to hypoxia, oxidative stress and inflammatory cytokines. The transient expression of HO-1 in MSC was able to induce anti-apoptotic and anti-oxidative stress mechanisms that increased their survival ability in vivo [116]. In different models of MI, it was also demonstrated that these cells could attenuate cardiac remodelling and increase angiogenesis through paracrine mechanisms mainly mediated by VEGF and $FGF_2$ [115, 118, 119].

Other methods to potentiate cell survival in vivo like Bcl2 or connexin-43 overexpression in MSC induced modest improvement in cardiac function (increase in ejection fraction around 4% relative to non-modified MSC) using greater numbers of infused cells [124, 125].

## Strategies to Improve MSC Paracrine Mechanisms

Genetic modification of MSC has also been directed to potentiate their paracrine effect (Table 4). In similar animal models to the ones described above, overexpression of growth factors like HGF, IGF or VEGF led to improvement in cardiac function, angiogenesis and reduction of infarct size [95, 103, 104, 106, 126–129]. However, the majority of these studies needed higher cell doses to induce beneficial effects than strategies directed to potentiate engraftment and survival. It is noteworthy that most of these genetic modifications led to activation of PI3K-Akt pathway both in MSC and transplanted hearts indicating that this is a pivotal signaling pathway for MSC mediated repair. For instance, administration of HGF or VEGF overexpressing MSC improved ventricular wall thickness, angiogenesis and cardiac performance of infarcted hearts [126, 127]. Calcineurin, phosphorylated Akt and Bcl-2 were significantly increased in HGF-MSC treated hearts [126]. In vitro, conditioned medium of HGF-MSC and VEGF-MSC protected hypoxia exposed murine cardiomyocytes reducing LDH release. Akt activity was also increased in cultured cardiomyocytes and correlated with a decrease in the apoptotic index, indicating that this molecule may also play a major role in cardiomyocyte survival [127]. In another study, overexpression of MiR-126 in MSC, an endothelial cell-specific miRNA, also resulted in increased levels of phosphorylated Akt in MSC and potentiated the capacity of MSC to induce angiogenesis in vivo [130].

Infusion of MSC overexpressing paracrine factors often induces pleiotropic effects. For instance, Tang and colleagues [102] demonstrated that infusion of SDF-MSC increased myocardial HGF expression levels in comparison with MSC treated hearts or control hearts. The same research group demonstrated that infusion of VEGF overexpressing MSC increased levels of myocardial SDF-1α [95]. IGF-1 overexpression in MSC also increased myocardial SDF-1α, phosphoinositide-3 kinase (PI3k) and Akt [104]. Moreover, as mentioned before, overexpression of the paracrine factors IGF-1 or VEGF in MSC resulted in stem cell homing to myocardium [95, 104]. Both VEGF and IGF-1 overexpressing MSC induced a massive c-kit+cardiac stem cell mobilization via SDF-1α signalling that culminated in increased angiogenesis in transplanted animals.

All these works show the interplay among SDF, VEGF, HGF and IGF in myocardial repair and corroborate the main contribution of paracrine mechanisms in MSC based therapies.

## Conclusion

Genetic engineering has managed to significantly increase engraftment, homing, survival and differentiation of MSC, therefore improving cardiac function in laboratory animals. Indeed, thanks to these approaches, we better understand some of the induced molecular pathways implicated in cardiac repair.

Several obstacles continue to impair the use of these cells in clinical trials. First of all, most of the studies were developed with murine or porcine MSC, thus we cannot discard differences in in vivo reactions when using human MSC. Second in, depending on the vehicle used genetic engineering is considered a risky approach for a clinical set-up. Thirdly, genetic modification strategy does not allow for a rapid treatment of the injured heart since it requires cell expansion and genetic modification of cells. In this context, MSC therapy should be done before the fibrous scar is formed since MSC contribute and potentiate the natural healing process, reducing infarct area and improving cardiac function. To overcome these problems, the use of drugs to stimulate MSC therapeutic mechanisms would be very helpful. Unfortunately, most drug treatments that stimulate these pro-survival and restorative pathways have not achieved the same results due to the transience of compounds in the body tissues. Thus, promising results are expected from the combination of cell therapy and controlled drug release fields.

Although it is still too early to justify a clinical strategy based in genetically modified MSC, several clues can guide research to improve therapy.

In general, strategies based of improvement on MSC differentiation are not supported enough mainly due to the limited degree of differentiation and impaired connection with healthy cardiac tissue. On the contrary, strategies to promote MSC survival showed significant improvement in cardiac function relative to treatments with wild type MSC, despite of the modest improvement in MSC engraftment and short term persistence of cells achieved in most cases with these genetic modifications.

Due to the difficulty to compare studies performed in different experimental conditions, we cannot conclude which of the many are the best strategies to potentiate MSC repair. It is necessary to perform comparative studies in the same experimental conditions to determine the most effective way to repair cardiac tissue, before going into the clinical setting [10]. Nevertheless, it is noteworthy that many genetic modifications potentiate MSC survival with the activation of the PI3K/Akt signalling pathway in vivo and in vitro, that is sufficient to trigger a significant restorative response based on paracrine mechanisms leading to angiogenesis and stem cell mobilization [86, 101, 112, 113, 117, 120, 122, 123, 125–127, 130–132].

Redundant mechanisms involving CXCR4/SDF-1α, HGF, IGF and VEGF appear to be also closely implicated in the repair process [95, 102, 104].

In summary, overexpression of survival molecules and growth factors appears to be a potent strategy to potentiate stem cell therapeutics. Exploring and dissecting the mechanisms triggered by MSC transplantation guarantee the improvement of MSC based therapies.

In this context, although we are far from this point, if we were able to achieve a clear improvement in cardiac function it would be possible to reach the clinic with genetically modified MSC and future cell based therapies could be developed, opening new horizons as banking of engineered MSC designed for healing different tissues and different pathologies.

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