Jürgen Hescheler · Erhard Hofer *Editors*

Adult and Pluripotent Stem Cells

Potential for Regenerative Medicine of the Cardiovascular System

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General Introduction

There is hardly an area of research developing so quickly and raising so much hopes and promises as stem cell research. Embryonic, fetal, and adult stem cells provide cells with pluri- and multipotency, respectively, and allow deeper insights into mechanisms of self renewal and plasticity. More recently, processes of epigenetic programming and reprogramming to induce pluripotent stem cells (iPS cells) hold the promise for better understanding the differentiation of embryonic stem cells into endo-, ecto- and mesodermal lineages as well as their derivatives. On the other hand, there is great hope that cells derived from stem cells in cell culture can be used for transplantation purposes and regeneration of diseased organs such as the heart (cardiac infarction), brain (Parkinson, neural trauma, stroke) and many other degenerative diseases. This book will concentrate on the perspectives of adult and embryonic stem cells differentiating into unique cell derivatives with a high capacity in regenerative medicine of cardiovascular diseases. The intriguing field of stem cell research under in vitro conditions in the embryoid body model combined with detailed functional characterization of the derived cells has originated in Germany by work of Wobus, Wallukat and Hescheler in 1991 (Differentiation 48:173–182). Since then more than 20 years have passed with a tremendous development of stem cell research. Due to the legal situation many researchers concentrate on fetal or adult stem cells and there are even several clinical studies on the effect of bone marrow derived stem cells after implantation into the infarcted heart. In order to cover this vast area and to bring together the state of the art and expertise a European Consortium INELPY has been formed in which protocols, data, ideas etc. were intensively exchanged to obtain a large surplus (http://cordis.europa.eu/projects/rcn/90957_en.html). This book disseminates the major outcome of this 3.5 years existing group and thus introduces the reader into this fascinating area of research and clinical application, but also incorporates very recent findings interesting for the expert reader. The book is unique as there is as yet no comprehensive overview on adult and embryonic stem cell based therapies. The book also provides high topicality as on the basis of induced pluripotent stem cells (iPS cells) a translation of basic research became possible and the current book will provide a conceptual basis for these future therapies.

The *In*farct C*el*l Thera*py* (INELPY) Consortium

The INELPY consortium was formed in 2009 to investigate different sources of adult and embryonic stem cells for their potential use to induce and support regenerative processes in ischemic heart disease. The consortium received support from the 7th Framework Programme of the European Commission from 2009 to 2012 (Health-F2-2009-222995: Therapy after myocardial infarction—repair by stem and progenitor cell transfer; see www.infarctcelltherapy.eu). The objectives of the project comprised the evaluation of selected cell preparations, the investigation of potential paracrine factors involved and novel application procedures to improve the effects on tissue repair.

The consortium included four laboratories active in basic and translational molecular stem/progenitor cell research with significant experience in animal infarct models, one group developing novel biomaterial technology and tissue engineering, two cardiology clinicians with experience in clinical cell therapies and two companies with portfolios to develop cell therapies and recombinant growth factors for stem cell growth.

The work performed focused on the one hand on progenitors of vascular endothelial cells and generation of blood vessels, since improved transfusion is the precondition for any repair processes in the damaged heart. On the other hand, the regeneration of heart muscle and heart function was investigated after application of various adult stem/progenitor cells or cardiomyocytes differentiated from embryonic stem cells. This included proof-of-concept studies in small and large animal models. Additional aspects were the improved release of progenitor cells from the bone marrow and the use of specific scaffold materials based on alginate to improve incorporation of the transplanted cells and paracrine effects of growth factors.

Major outcomes of the consortium project showed that a certain type of circulating endothelial progenitor cells, i.e. ECFCs/BOECs, leads to the best long-term engraftment observed for any of the progenitor cells tested, contributed to neovascularization and improved perfusion. As increased perfusion is the precondition for any repair and regrowth of myocardium, these data suggest that intracoronary application of these cells could become a first step therapy to improve perfusion. This could then be followed or combined with therapies designed to replace damaged cardiac tissue by regrowth of heart muscle. In this regard, data from the consortium

show that transplantation of stem cells from bone marrow (MAPCs and MSCs) as well as adipose tissue (ADSCs) induce a functional benefit in cardiac tissue despite a very limited degree of long-term engraftment (see contribution by M. Mazo et al.). The mechanisms by which these cells contribute to cardiac repair are presumably to a large extent related to the release of paracrine factors. Some clinical data using BM-derived MSCs have been already obtained by one of the consortium partners (see contribution by J. Kastrup et al.).

Importantly, in regard of proteins/genes involved, we have defined two novel factors which seem to be responsible for at least part of the provascularization functions of ECFCs/BOECs (see contribution by R. Hofer-Warbinek et al.). The data suggest that these factors are responsible for at least part of the positive effects of these cells on perfusion and could be used to support provascularization. Furthermore, the potential of ADSC to contribute to vascularization is described by P.C. Baer and W. Luttmann.

For ex vivo generation of cardiomyocytes significant progress has been achieved (see contribution by B. Krausgrill, M. Halbach and J. Hescheler). Cardiomyocytes have been efficiently generated from embryonic or induced pluripotent stem cells. However, their use in transplantation is still hampered by the low incorporation rate achieved when applied in animal models and the need for correct electrophysiological coupling to the endogenous heart muscle, issues which still have to be improved.

Data using different preparations of biomaterials have further shown that transplantation of cells together with alginate biomaterials improve cell engraftment and function (see contribution by E. Ruvinov and S. Cohen). In addition, significant hopes for future therapies are based on the development of prevascularized patches of heart muscle grown ex vivo from mixtures of stem and/or differentiated cells. In this project we have made significant progress by engineering alginate scaffolds fort his purpose and the application of various electric and magnetic stimulation patterns during cell growth.

In the following contributions of this compendium partners of this consortium review the state-of-the-art of their specific field and summarize their data obtained in the course of the INELPY project. Moreover, S. Janssens and J. Kastrup and collaborators give an overview how the available experimental data from animal models have already resulted in first clinical trials and how these will be further developed in the near future to improve clinical cell therapy of ischemic cardiomyopathy after myocardial infarction.

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Chapter 1
Mesenchymal Stem Cells for Cardiac Repair: Preclinical Models of Disease

Manuel Mazo, Miriam Araña, Beatriz Pelacho and Felipe Prosper

Abstract In recent years, the incredible boost in stem cell research has kindled the expectations of both patients and physicians. Mesenchymal progenitors, owing to their availability, ease of manipulation and therapeutic potential, have become one of the most attractive options for the treatment of a wide range of diseases, from cartilage defects to cardiac disorders. Moreover, their immunomodulatory capacity has opened up their allogenic use, consequently broadening the possibilities for their application. In this review, we will focus on their use in the therapy of animal preclinical models of myocardial infarction, with special focus on their characteristics and their in vitro and in vivo mechanisms of action.

Introduction

In spite of the increasing knowledge of its causes, development and treatment, myocardial infarction (MI) remains the greatest health concern worldwide (Organization WH 2008). Following the ischemic event, the damage inflicted upon cardiac cells rapidly becomes irreversible, leading to massive death and loss not only of muscular but also vascular cells (Mazo et al. 2010b). Although clinical practice has raised the expectancy of surviving an MI and is able to mitigate its progression (White and Chew 2008), the mammalian (and therefore the human) heart lacks a significant capacity to self-heal, leaving a chronically scarred organ with a limited and waning functionality, which will finally lead to heart failure and the dichotomy of transplantation versus death.

During the last 15 years, the boost in stem cell research has ignited the expectations of patients and scientists alike. Although the initial hypothesis was that these plasticity-endowed cells could mend what disease had broken through direct replacement of lost cell populations (Reinecke et al. 2008), an increasing body of evidence points towards their capacity to secrete therapeutic molecules as a potent regenerative tool (Gnecchi et al. 2005). Among the different cell types assayed, mesenchymal stem cells (MSC) are one of the most interesting and intensively

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investigated. In the following pages we will revise what characteristics make mesechymal progenitors so appealing for cardiac regeneration as well as discuss the available data from studies in which MSC have been employed to treat animal models of MI.

Origin, Characteristics and Isolation of MSC

In 1974, Friedenstein et al. published one of the first reports on MSC (Friedenstein et al. 1974a, b), demonstrating the clonogenic potential of multipotent cells in the marrow, which they termed colony forming unit-fibroblasts. Since then, our knowledge of these cells has greatly increased, and MSCs are now viewed as a mesoderm-derived population, ubiquitously found in a variety of tissues, most abundantly bone marrow (BM) and adipose tissue (AT), but also in dental pulp, menstrual and cord blood or placenta, among other sites (Mazo et al. 2011; Bianco et al. 2008; Gandia et al. 2008; Hida et al. 2008; Lee et al. 2004; Yen et al. 2005). Nevertheless, their physiological function is still not well established, mainly due to the lack of an appropriate marker for their in vivo identification and the difficulty of performing adequate lineage-tracing experiments. Although the possibility of their residence within the proximity of vessels has been hinted (Cai et al. 2009b), this has not been extensively proved. It is widely assumed, however, that MSCs give support and nurture to other cells, with several studies identifying their role in endogenous tissue repair processes (reviewed in Caplan and Dennis 2006).

This plethora of origins and capacities has impelled standardization, as the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed in 2006, concluding that the minimum requirements to define MSC (Dominici et al. 2006) are: first, cells must be plastic-adherent under currently established culture conditions; second, MSC should express CD73, CD90 and CD105, and lack expression of HLA-DR, CD11b, CD14, CD19, CD34, CD45 or CD79-alpha; finally, they must be able to differentiate to osteoblasts, chondrocytes and adipocytes in vitro. Still, caution must be taken as some reports fail to meet these criteria, and MSC is often employed for “Marrow Stromal Cell”, “Mesenchymal Stromal Cell” or “Marrow Stem Cell”. Accordingly, a clarification was published in which MSC was defined as “Multipotent Mesenchymal Stromal Cells” (Horwitz et al. 2005), adding the supportive property to the required characteristics (Sacchetti et al. 2007). Nevertheless, tissue of origin gives isolated progenitors a distinct therapeutic potential, making comparisons between different populations difficult. For example, the relative abundance of mesenchymal progenitors in BM versus AT (0.01 %–0.0001 % of cells in the marrow (Pittenger et al. 1999) and 100 to 500 times that number in adipose depots (Mazo et al. 2011) may importantly impact cell growth kinetics and other characteristics, as has been proved at a functional, genomic and proteomic level (Wagner et al. 2007; Kern et al. 2006), suggesting a higher degree of commitment of BM-MSC to chondrogenic and osteogenic lineages than adipose-derived MSC (termed adipose-derived stem cells,

ADSC) (Gimble et al. 2007). Placenta-derived mesenchymal cells share many of the characteristics of MSC, but have been shown also to express embryonic stem cell antigens as SSEA-4, Tra-1-61 or Tra-1-80 (Yen et al. 2005), whereas umbilical cord-MSC (Lee et al. 2004) represent a more juvenile source of cells, not subjected to patient specific constrains as the negative influence exerted by of age or disease (Dimmeler and Leri 2008). Moreover, one of the most appealing characteristics of MSC is their capacity for suppressing the host immune response, but this has only been well established for BM-MSC (Le Blanc et al. 2008). In general however, it is not clear whether the mentioned differences are due to true divergences in the nature of the progenitor populations or to the in vitro processing. The tissue of origin clearly influences the properties of the cells, as already remarked for the case of cord blood. Head-to-head comparisons would allow the solving of this problem, but they remain a scarce investment (Luan et al. 2013; De Ugarte et al. 2003).

The isolation of mesenchymal progenitors is nowadays a straightforward technique, which commonly relies on obtaining an intermediate mixture of progenitors. When the starting sample is BM or blood, a Ficoll gradient centrifugation or similar process is employed to remove most contaminating erythrocytes (Hida et al. 2008; Chang et al. 2008; Mazo et al. 2010a, b), rendering the mononuclear fraction. AT processing requires careful mincing plus an enzymatic (usually collagenase) digestion of the specimen to segregate adipocytes from the stromal vascular fraction (Mazo et al. 2008). In the case of term placenta, after draining of any remaining cord blood, the tissue is similarly minced and digested. In vitro culturing under similar conditions, gives rise to the purified MSC populations, be it from the mononuclear fraction (for BM and cord blood MSC) or from the digested tissue (for AT and placenta). Thus, unless allogenically employed, the use of mesenchymal progenitors implies several weeks of processing prior to their application to the patient. This issue will be further discussed below, as it has fundamental implications for their use in the cardiovascular field.

The Triple Goal of Cardiac Regeneration and MSCs

The reconstruction of the diseased left ventricle (LV) is a final goal with three main aims: (1) the regeneration of the cardiac muscle (Rogers et al. 2011), (2) the formation of a mature, functional vascular network to irrigate it and (3) the return of the damaged tissue to its former geometry (Dixon et al. 2009). Failure in accomplishing any of the three would impair the outcome of the therapy. Stem cells, and mesenchymal progenitors among them, can contribute to these mainly through two mechanisms: certain differentiation to the desired phenotypes and, of more significance, releasing of molecules with therapeutic potential. Further increasing their capacities, mesenchymal cells have also the ability to regulate the immune response, which further widens their possible applications (Fig. 1.1).

Transdifferentiation of MSC to the desired phenotypes has been documented. BM-MSC have shown their capacity to give rise either to endothelial cells (EC)

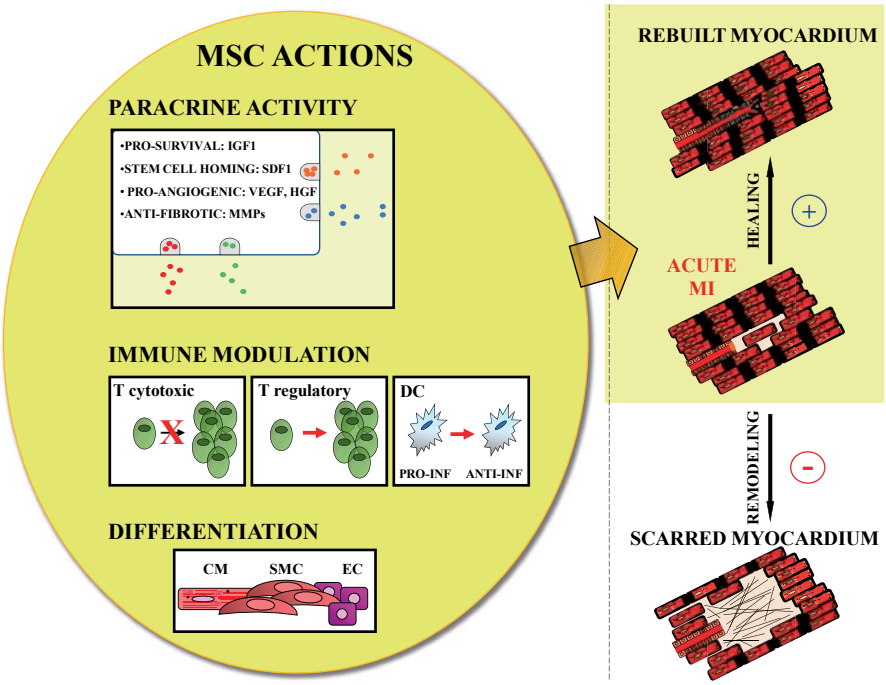

Fig. 1.1 MSC actions on injured myocardium. Mesenchymal progenitors transplanted onto the ischemic myocardium influence infarct healing through three main processes: *i* the secretion of therapeutic molecules (paracrine activity), *ii* the modulation of the immune response, promoting an anti-inflammatory state and *iii* giving rise to differentiated (cardio)vascular cells, encouraging the healing of the damaged tissue and avoiding its transition to a scarred muscle. Abbreviations: *IGF-1* insulin-like growth factor-1, *SDF-1* stromal derived factor-1, *VEGF* vascular endothelial growth factor, *HGF* hepatocyte growth factor, *MMP* matrix metalloproteinase

(Lozito et al. 2009; Liu et al. 2007) or smooth muscle cells (SMC) (Le Blanc et al. 2008), but the obtaining of cardiomyocytes (CM) has been contested due to the immaturity of the resulting cells (Yan et al. 2011; Arminan et al. 2010) or the use of demethylating agents (Xu et al. 2004). Similarly, although ADSC are able to differentiate into vascular cells (Kim et al. 2008; Planat-Benard et al. 2004), the obtaining of CM has been accomplished under treatment with DMSO (van Dijk et al. 2008). Other types of MSC have also been differentiated to CM or CM-like cells, such as menstrual blood-derived MSC (Hida et al. 2008) or umbilical cord-MSC (Chang et al. 2008). In recent years AT has been proposed as a much richer source of stem cells with cardiac and endothelial potential than the BM, yet a cautionary note must be risen as these studies either rely on freshly isolated cell subpopulations or they culture cells under differentiating promoting conditions without true prior mesenchymal enrichment (Leobon et al. 2009; Sengenes et al. 2007), so direct comparison between those and BM-MSC is not possible. To date, though angiogenic potential of MSC is better established and is probably related to the pericytic nature

of these cells (Cai et al. 2009b; Jain et al. 2008), their differentiation into cardiac myocytes is still heavily challenged.

However, the suitable exploitation of the differentiation capacity of MSC in MI still presents certain caveats. First, patients receiving stem cell therapy are severely diseased and usually elderly, two factors that have an outstanding impact on stem cell function. For instance, a decrease in the numbers and functionality of circulating endothelial progenitors is directly related to cardiovascular risk and smoking (Kondo et al. 2004; Vasa et al. 2001) and age has also been shown to impair the angiogenic capacity of both BM-MSC and ADSC (Liang et al. 2011; Madonna et al. 2011). Second, cell engraftment is poor, with few cells capable of long-term persistence (reviewed in Haider and Ashraf 2008) in the scarred myocardium. Third, the loss of a significant mass of contractile cells and their associated vessels imposes a burden that is almost impossible to overcome. The myocardium hosts on average 20 million cardiomyocytes (CM) per gram of tissue which, considering a human left ventricle (LV) of about 400 g, means that in cases when an MI damages 25 % of it, about 1 billion contractile cells are lost, as well as other components of the cardiac architecture (fibroblasts, endothelial and smooth muscle cells, etc.) (Robey et al. 2008). Thus all of these factors, coupled to the low rates of cell differentiation achieved even under in vitro controlled conditions, make the rebuilding of the LV through the addition of such a small number of cells a rather naïve strategy.

On the other hand, MSC have been shown to be first-class paracrine secretors, being able to induce a significant effect upon the damaged heart, even when low numbers of cells manage to engraft (Fedak 2008). Angiogenic molecules like vascular endothelial growth factor (VEGF) or hepatic growth factor (HGF) have been shown to be secreted by either BM-MSC or ADSC (Oskowitz et al. 2011; Traktuev et al. 2008; Chen et al. 2008b; Kilroy et al. 2007; Song et al. 2007). Additionally, ADSC have been reported to form and stabilize functional vascular networks when mixed with endothelial progenitors (Traktuev et al. 2009) and Chen et al. showed that BM-MSC were a more potent source of pro-angiogenic cytokines than dermal fibroblasts, exerting a stronger effect upon the recruitment of EC and macrophages and improving wound healing (Chen et al. 2008b). The role of mesenchymal progenitors such as fibrosis-regulators is similarly extensively documented. Conditioned medium from BM-MSC decreases cardiac fibroblast proliferation and modifies the secretory equilibrium towards an anti-fibrotic milieu, with diminished expression of collagen types I and III (Li et al. 2009b; Ohnishi et al. 2007) and increased secretion of matrix metalloproteinases (MMP) 2, 9 and 14 (Mias et al. 2009). Likewise, ADSC produce transforming growth factor (TGF) β1, one of the most potent regulators of fibroblast behavior and fibrosis (Rehman et al. 2004). Beside perfusion promotion and collagen architecture regulation, MSC are able to migrate to the sites of injury due to their expression of the chemokine receptor CXCR4 (Cheng et al. 2008b) and traffic through type I collagen membranes as demonstrated by the expression of five types of MMPs (2, 3 and membrane type-MMPs 1, 2 and 3) (Rogers et al. 2011). These two features would theoretically allow MSC to home to and migrate through the scarred myocardium.

Taken as a whole, all of these exemplify the plethora of paracrine-mediated actions that mesenchymal progenitors can set into motion.

As already noted, immune regulation is one of the most interesting competences of MSC (reviewed in Le Blanc 2006). Marrow-derived MSC inhibit the proliferation of activated T cells and the formation of cytotoxic T cells (Aggarwal and Pittenger 2005), inducing an anti-inflammatory phenotype. This unique capacity significantly broadens the scope of MSC use, as it opens the way to allogenic applications or even to their use as "inducers of tolerance" to other cell types (Bel et al. 2010). However, some concerns have been reported. Huang et al. reported that differentiation reduced the capacity of immunological escape of BM-MSC (Huang et al. 2010b), related to an increase in immunostimulatory molecules MHC-Ia and II and a decrease in the immunosuppressive MHC-Ib. Along similar lines, McIntosh and coworkers reported that ADCS beyond passage 1 (and thus devoid of contaminating differentiated cells) (Mitchell et al. 2006) failed to elicit a response from allogenic T cells (McIntosh et al. 2006), but this attribute may be diminished under inflammatory stimuli, as shown in vitro (Crop et al. 2010). As a consequence, the true ability of MSC to be tolerated by the host's immune system is still debated, and has proved not so significant as initially thought (Puymirat et al. 2009).

Finally, since the groundbreaking report in 2006 by Yamanaka and coworkers (Takahashi and Yamanaka 2006) where induction of pluripotency in adult (stem) cells by overexpression of the pluripotency transcription factors Oct3/4, Sox2, Klf4 and c-Myc was shown, mesenchymal cells have been investigated intensively (Tat et al. 2010; Sun et al. 2009). Advantages like the ease of harvest and culture together with a higher potency than other cell types (e.g.: dermal fibroblasts), has made them good candidates for iPS derivation.

MSC in Preclinical Models of MI

The assessment of stem cells in animal models of MI is a compulsory test before any further step is taken. Results from clinical trials that have already finished have demonstrated the critical impact that not only the treatment, but most importantly the type of disease where cells are applied on, has on the outcome of the study (reviewed in Menasche 2011). Although cardiovascular diseases have been widely modeled, stem cell therapy has been mostly centered on permanent/transient models of myocardial ischemia, with few reports dealing with other settings (de Macedo Braga et al. 2008). However, a distinction between three different models can be made: acute, sub-acute and chronic models of MI.

In the acute form, cells are transplanted within hours of the induction of ischemia. Here, cell therapy must cope with a pro-inflammatory microenvironment and the necrotic/apoptotic molecules released from dying cells (Nian et al. 2004; Mann 1999). Nevertheless, both the presence of homing signals and an anti-fibrotic milieu can be a positive counterbalance (Penn 2009; Cleutjens et al. 1995). The great majority of published reports has relied on this model (Chang et al. 2008; Ii et al. 2011;

Gaebel et al. 2011; Bai et al. 2010, 2011; Dubois et al. 2010; Yang et al. 2009; van der Bogt et al. 2008, 2009; Dixon et al. 2009; Imanishi et al. 2008; Hashemi et al. 2008; Hale et al. 2008; Carr et al. 2008; Cai et al. 2009a; Valina et al. 2007; Li et al. 2007) due to the fact that transplanting cells at the time of coronary occlusion (or minutes/hours after it) subjects animals to only one operation and consequently decreases the mortality associated with the process. With the exception of studies by van der Bogt et al. (van der Bogt et al. 2008, 2009), all specialists have consistently demonstrated that therapy with MSC induces a significant benefit upon cardiac function, which has mainly been attributed to the paracrine activity of the cells that induces an increase in tissue perfusion and a decrease in the size of the scar and collagen content.

In spite of these results, implementation of a therapy based on transplanting mesenchymal progenitors (a population that needs several weeks of in vitro purification) on the acute phase of the disease is not possible unless allogenically employed. Strikingly, far fewer studies have dealt with the opposite situation, the chronic phase (Mazo et al. 2008, 2010a; Song et al. 2010; Lee et al. 2009), where the repair processes take place after the ischemia has been completed: inflammation has receded, the scar has matured and although a new vascular network has been developed, it is disorganized and insufficient (Virag and Murry 2003; Sun et al. 2002). Notwithstanding the great burdens that these conditions impose upon transplanted cells, the abovementioned studies have demonstrated that, as shown for the acute phase, MSCs exert a positive action on cardiac contractility and histology, again mostly due to the secretion of therapeutic molecules and the action on resident cells.

Finally, a third, intermediate setting can also be found: the sub-acute. In this, angiogenic processes are still on course, either through endothelial progenitors (Jujo et al. 2008) or macrophages (Nahrendorf et al. 2007), and the receding of inflammation is coupled to an increase in fibrotic processes. Again, reports in models of sub-acute MI are scarce (Gandia et al. 2008; Hida et al. 2008; Wang et al. 2009a; Amado et al. 2005), but the benefit and mechanisms, as in acute and chronic models, appear to be consistent.

Thus, a fair amount of information is available, supporting the capacity of mesenchymal progenitors to exert a benefit on cardiac function. The mechanistic basis of this is not yet fully explained and it seems to be far more complex than what was expected. However, it is widely accepted that MSC exert their influence upon cardiac contraction not by directly resupplying the tissue with new CM, but through more subtle actions on organ architecture and histology. Some of the abovementioned studies provide crucial hints. Lee and coworkers showed that transplantation of BM-MSC in a rabbit model of chronic MI induced an increase in the concentration of stromal derived factor (SDF) 1, which elicited the chemotaxis of host-derived BM progenitors (CD34+, CD117+, STRO1+) and was related to a functional benefit, a decrease in infarct size and improvement in tissue vascularization (Lee et al. 2009), as similarly shown by Suzuki et al. in a swine model of hibernating myocardium (Suzuki et al. 2011). Li and collaborators demonstrated that the enhanced contractility was accompanied by a preservation in the pro-survival signaling of Akt (Li et al. 2009a), whereas Mias et al. showed that this functional

improvement and remodeling reversal in vivo was coupled to a myriad of in vitro anti-fibrotic actions (Mias et al. 2009). The Spinale group monitored the evolution of MMPs and their inhibitors after infusion of BM-MSC in a model of sheep MI, establishing a relationship between the number of transplanted cells and enzymatic levels (Dixon et al. 2009). Resembling what was found in vitro, several reports have associated the pro-angiogenic activity of the cells with the secretion (either directly or host-derived) of potent angiogenic cytokines such as VEGF, HGF or insulin-like growth factor (IGF) 1 among others (Gandia et al. 2008; Li et al. 2007, 2009a; Wang et al. 2009a, b).

The Problems and Their Solutions

Despite the initial optimism, the fire that fueled the field in its beginnings has cooled down. Although positive, results from clinical trials have not been as good as it had been anticipated (reviewed in Menasche 2011) and the desired goal of replacing the scar with a functional myocardium has not yet been achieved. Cell therapy presents certain caveats that need to be improved before the goal of regeneration is achieved. There are two main difficulties: lack of efficient differentiation and low survival/engraftment capacity. As one of the spearheads of cell therapy, MSC are currently undergoing intense investigation to solve both of these issues (Fig. 1.2).

The question of improving cell survival and permanence at the site of injury has been dealt with by two chief approaches: cell modification and tissue engineering. Albeit with certain constraints, genetic manipulation of MSC has undergone profound investigation. Among the many possibilities, the CXCR4/SDF1 axis has been shown to have a capital role in cell trafficking and homing. As a consequence, many reports have exploited it, trying to improve the therapy. Ma et al. investigated the peak of cardiac SDF1 expression after MI in rat, showing that injecting cells at that time point (1 day post-infarction) significantly enhanced cell engraftment and angiogenesis (Ma et al. 2005). Cheng and collaborators injected BM-MSC engineered to overexpress the receptor CXCR4, strengthening cell-homing to the injured tissue (Cheng et al. 2008b). The same group combined BM-MSC peripheral injection with administration of granulocyte colony-stimulating factor (G-CSF). Although G-CSF was able in vitro to increase CXCR4 expression and in vivo to augment cell engraftment, no effect on cardiac function was found (Cheng et al. 2008a). Recently, the group led by Dzau showed that overexpression of the chemokine receptor CCR1, but not CCR2, was associated with improved survival and grafting in a mouse model of MI, as well as functional restoration (Huang et al. 2010a).

Similarly, the issue of cell survival has been dealt with through genetic approaches. Liu et al. engineered BM-MSC to overexpress angiogenin, which conferred an improved resistance to hypoxia and was translated into an increase in cell engraftment and functional/histological recovery (Liu et al. 2008). Adenoviral-transfection of hemeoxygenase-1 resulted in a superior therapeutic effect, mainly through protection from inflammation and apoptosis (Zeng et al. 2008). Akt-overproduction in