Published in final edited form as:

Basic Res Cardiol. 2010 July ; 105(4): 443–452. doi:10.1007/s00395-010-0102-7.

Hepatocyte growth factor/Met gene transfer in cardiac stem cells —potential for cardiac repair

Rosalinda Madonna,

Institute of Cardiology, Center of Excellence on Aging, “G. d’Annunzio” University Chieti, Via dei Vestini, 66013 Chieti, Italy

Gregg Rokosh,

Institute of Molecular Cardiology, University of Louisville, Louisville, KY, USA

Raffaele De Caterina, and

Institute of Cardiology, Center of Excellence on Aging, “G. d’Annunzio” University Chieti, Via dei Vestini, 66013 Chieti, Italy

Roberto Bolli

Institute of Molecular Cardiology, University of Louisville, Louisville, KY, USA

Rosalinda Madonna: rmadonna@unich.it

Abstract

The adult heart has been recently recognized as a self-renewing organ that contains a pool of committed resident cardiac stem cells (CSCs) and cardiac progenitor cells (CPCs). These adult CSCs and CPCs can be induced by cytokines and growth factors to migrate, differentiate, and proliferate in situ and potentially replace lost cardiomyocytes. Ligand-receptor systems, such as the tyrosine kinase receptor mesenchymal–epithelial transition factor (Met) and its ligand hepatocyte growth factor (HGF), are potential candidates for boosting migration, engraftment and commitment of CSCs. Here, we discuss the possible application of HGF/Met gene therapy to enhance the ability of CSCs to promote myocardial regeneration.

Keywords

Cardiac stem cells; Cardiac progenitor cells; Cell-based therapy; Hepatocyte growth factor; Gene therapy; Cardiac repair

Introduction

Injury to the myocardium, such as that occurring as a consequence of myocardial infarction, is a major cause of death, as the human heart has a limited capacity to regenerate. Possible approaches to treat the late consequences of myocardial damage, i.e., heart failure, include transplantation of stem cells into the heart. Several types of stem cells have been used so far to repopulate functional myocytes or vascular cells in the damaged myocardium, including cardiac stem cells (CSCs) and cardiac progenitor cells (CPCs) [12, 13], endothelial progenitor cells (EPCs) [26, 35, 55, 57], bone marrow-derived stromal/stem cells [1, 22, 38, 46, 59] and adipose tissue-derived stromal/stem cells [10, 31, 34, 36, 39, 56, 63, 70, 72]. In most studies, stem cell therapy has proven to provide beneficial increases in function,

although mechanisms underlying this improvement are unclear. Besides differentiation into cardiomyocytes, these approaches likely involve altered remodeling of the vasculature, scar and extracellular matrix and other poorly defined paracrine effects. Few studies have indeed clearly demonstrated stem cell differentiation into functional adult cardiac myocytes [20, 25, 53, 54, 74]. In vivo and in vitro studies employing bone marrow-derived stem cells have actually shown that newly differentiated myocytes from such undifferentiated cells do not acquire the adult phenotype of cardiomyocytes, but rather resemble neonatal cells, which die over time by apoptosis [20].

Recently, the seminal observation that the adult heart possesses a pool of resident CSCs (which are self-renewing, clonogenic and multipotent stem cells) and CPCs has suggested a different approach to myocardial repair, i.e., to facilitate myocardial regeneration by inducing endogenous cardiac cells to migrate, differentiate, and proliferate in situ to replace lost cardiomyocytes [6]. Access to tissue for the isolation of CSCs and CPCs can be obtained from patient myocardium via atrial appendage harvest during bypass surgery (SCIPIO Trial) or by endomyocardial biopsy making use of these cell populations feasible for cell therapy. Despite this, there is still significant controversy as to whether or not CSCs differentiate into new blood vessels or new myocytes.

While the best cell type, optimal timing, and route for transplantation still need to be determined, there has been increasing attention toward novel pharmacological and genetic strategies to enhance the implantation of genetically modified stem cells or for boosting endogenous regeneration of myocardial tissue. Pharmacological agents or genetic modification may enhance the efficacy of cell therapy in several ways: mobilizing endogenous stem/progenitor cells in vivo; promoting cell proliferation and differentiation; and augmenting cell engraftment and survival in the injured myocardium. In this context, a major challenge is presented by the identification of growth factors (GFs) and signaling pathways that selectively promote resident CSC proliferation, migration, engraftment, and differentiation [2, 29], the understanding of which might open new prospects for stem cells in cardiovascular repair and regeneration.

Recent studies [32, 62] have reported the in vivo and in vitro abilities of cytokines and GFs, such as insulin growth factor (IGF) and hepatocyte growth factor (HGF), to promote the translocation of CSCs into the injured area and to activate their growth and differentiation, resulting in the formation of functionally competent myocardium. Indeed, Anversa and colleagues [32, 62] have recently reported that the intramyocardial injection of HGF and IGF stimulates the translocation of resident CSCs into the infarct area from the surrounding myocardium and promotes the formation of new myocardium, leading to improvement in local wall motion and overall ventricular function in mice and dogs. The property of HGF to induce translocation of CSCs has been attributed to the expression of matrix metalloproteinases (MMPs) [23], which digest collagen and other extracellular components of the interstitium, facilitating cell migration and homing in the heart [62]. Indeed, the inhibition of MMP-2/MMP-9 significantly abrogated the invasive properties of CSCs stimulated by HGF [32]. Based on these observations, the authors suggested that local administration of GFs, such as HGF, may become a novel powerful therapeutic strategy for repairing the infarcted heart [62].

Here, we will review key concepts and mechanisms of signal transduction that support a role for the HGF/Met system in myocardial regeneration. Here, we will discuss the potential therapeutic use of HGF and Met via a gene therapy approach to enhance CSC function and to target the tissue environment during cell transplantation.

The HGF/Met system: structural features

HGF, also named scatter factor (SF), was first discovered for the independent properties of HGF and SF, through its ability to induce hepatocyte growth in culture [9] and scatter epithelial cells, i.e., of inducing the dissociation of epithelial cells in culture, letting them assume mesenchymal cell morphology and motility [67]. Subsequently, both these properties were associated with the same factor [66]. HGF/SF (subsequently referred to as HGF) is a large, multidomain protein similar to plasminogen, consisting of six domains: an amino-terminal domain (N), four kringle domains (K1–K4), and a serine proteinase homology (SPH) domain, which lacks enzymatic activity as a result of mutations in essential amino acid residues. Like plasminogen, HGF is synthesized as an 82-kDa single-chain, largely inactive precursor (pro-HGF/SF), and is converted proteolytically into a two-chain, active heterodimer. Cleavage of pro-HGF occurs at a trypsin-like site that is located after K4, and this produces a disulphide-linked α - and β -chain heterodimer [7].

The HGF receptor Met is a disulphide-linked heterodimer, which results from cleavage of a precursor (170 kDa) at a site located between amino acid residues 307 and 308. The mature form of Met consists of an extracellular α -chain (50 kDa) and a longer β -chain (140 kDa). The β -chain encompasses the remainder of the Met ectodomain, the transmembrane helix and the cytoplasmic portion. The latter contains the juxtamembrane and kinase domains, as well as a carboxy-terminal tail that is essential for downstream signaling. The juxtamembrane region contains several negative regulatory sites, which are involved in the downregulation and/or degradation of the receptor, corresponding to the site of caspase-mediated cleavage of Met. The α -chain and the first 212 amino acid residues of the β -chain are sufficient for HGF binding [7] (Fig. 1).

Cell targets and signaling pathways of the HGF/Met system

Upon HGF binding, Met dimerizes, and its tyrosine kinase activity is stimulated, resulting in the autophosphorylation of the receptor at tyrosine residues Y1349 and Y1356 [9]. Phosphorylated Met binds intracellular substrates, such as growth factor receptor-bound protein (Grb)-associated binder (Gab)1, Grb2, and phosphatidylinositol-3-kinase (PI3K). Steric hindrance prevents two substrates from binding simultaneously to one Met molecule. However, Gab1 can bind to phosphorylated (p)Y1349 on one, and Grb2 can bind to pY1356 on a second Met molecule. Therefore, an interaction between Gab1 and Grb2 may occur on Met dimers or multimers. Most of these Gab1 and Grb2 molecules contain binding sites for Src homology-2 (SH2)-domain, which mediate binding to SH2-domain-containing substrates. Substrates for phosphorylated Gab1 are protein tyrosine phosphatase 2 (Shp2), phosphoinositide 3-kinases (PI3K), and phospholipase C. Phosphorylated Grb2 binds to ras and raf, which in turn activates the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK) [9]. In response to Met signaling, proliferative and anti-apoptotic responses are evoked. Typical cell targets for HGF are hepatocytes, renal tubule cells, and endothelial cells, where HGF induces proliferation [18]. In addition, HGF/Met signaling can induce several different epithelial and mesenchymal cell types to undergo a differentiation program termed “branching morphogenesis”. This results in the creation of branching patterns leading to the formation of many essential organs—the lung, vascular system, and most glands—composed of ramifying networks of epithelial tubes that transport fluids [8, 24]. In vivo, Met expression is predominantly found in cells of epithelial origin, while HGF expression is usually restricted to fibroblasts and stromal cells in the surrounding mesenchyma [27]. Paracrine signaling between HGF and Met is believed to play an important role in regulating these epithelial–mesenchymal cell interactions.

In vivo, HGF and Met likely play a key role in regulating many aspects of embryonic development, including kidney and mammary gland formation, migration and development of muscle and neuronal precursors, as well as liver and placenta organogenesis [24]. HGF/Met signaling also promotes angiogenesis [5, 42], and has been described to facilitate wound healing and tissue regeneration [43]. The PI3K pathway is involved in HGF/Met-mediated morphogenesis and survival. It has indeed been shown that Gab1 regulates the orientation of PI3K to these different responses [19]. The overexpression of Gab1 inhibits HGF-mediated survival, likely as a consequence of inhibition of the sustained activation of Akt, whereas Gab1 overexpression promotes HGF-induced morphogenesis. The survival responses triggered by HGF are favored by the direct binding of PI3K to Met, leading to sustained activation of Akt, whereas recruitment of Gab1 directs HGF signaling toward morphogenesis. In response to HGF, Akt phosphorylates the proapoptotic protein Bcl-2-associated death promoter (BAD), leading to its inactivation and thereby preventing mitochondrial-dependent apoptosis [69]. Furthermore, survival responses induced by HGF are correlated with an increased expression of the anti-apoptotic Bcl-xL and Bcl-2 proteins, which again prevents mitochondrial-dependent apoptosis. Mitochondrial-dependent apoptosis depends on the release of cytochrome *c* from the mitochondria and the formation of the apoptosome, leading to caspase-9 activation and the subsequent activation, by cleavage, of caspase-3 [69].

Proliferation and survival responses to HGF also involve the ras-ERK pathway, through the binding of Met to Grb2 and the subsequent activation of the transcription factor nuclear factor- κ B (NF- κ B) [8]. Epithelial cells respond to HGF/Met signaling by ‘scattering’, i.e., undergoing colony dispersal and an increase in motility. Such dissociated cells thus invade collagen matrices. This phenomenon is used in an assay to estimate the invasive and metastatic capacity of cells. Moreover, when epithelial cells are cultured within a collagen matrix and treated with HGF, they form branched tubules. Tubular branching is a complex morphogenic process that is observed in culture, and requires a tight coordination of cell growth, cell–cell contacts, polarity, and movement. It has indeed been shown that HGF modulates cell–cell contacts and therefore tubular branching through a reorganization of the cytoskeleton [52]. For example, cadherin proteins form the core of *adherens* junctions, but become relocalized and randomly distributed in the cell membrane during stimulation with HGF [47]. β -Catenin, another *adherens* junction component, associates with E-cadherin, and then binds a third protein, α -catenin, to the cytoskeleton. HGF stimulation results in the tyrosine phosphorylation of β -catenin, which induces its dissociation from the cadherin complex [41].

Initial experiments showed that inhibitors of PI3K or ERK prevent the scattering of epithelial cells and cell–cell contacts, and this indicates that both the ERK and the PI3K pathways are important for the disassembly of *adherens* junctions, cell spreading and motility. The Gab1-Shp2-ERK cascade regulates transformation-specific sequence/activator protein 1 (ETS/AP1) transcription factors, as well as adhesion molecules, which control cell proliferation, junctional competence and motility [7] (Fig. 2).

Effects of HGF/Met on the commitment of CSCs: in vitro studies

HGF has been shown to be a potent differentiating factor for human embryonic stem cells (ESC), as well as for rat bone marrow mesenchymal stem cells (MSC) [44]. HGF and its receptor Met are expressed not only in fully differentiated cardiac cells, but also in myocytes during early cardiogenesis. Based on this observation, it has been speculated that HGF might be involved in cardiac development [49]. Forte et al. [17] have recently demonstrated the involvement of HGF in the in vitro cardiac commitment of murine MSC. After 2 days of treatment with HGF (20 ng/mL), MSC started to express transcription factors for muscle

differentiation and early cardiac development, such as myocyte enhancer factor (MEF)-2C, transcription enhancer factor (TEF)-1 and guanine/adenine/thymine/adenine (GATA)-4 binding protein, as well as cardiac contractile proteins, such as α -myosin heavy chain (MHC) and β -MHC, while losing stem cell markers such as nucleostemin, CD105, and c-kit [17]. Roggia et al. [50] have provided evidence that exogenous HGF can improve cardiac commitment of differentiating ESC through the upregulation of transcription factors for early cardiac development such as Nkx 2.5 and GATA-4. After HGF treatment of ESC, these authors showed an upregulation of markers for differentiated cardiomyocytes, such as tropo-nin I, α -MHC and β -MHC. These effects were mediated by the PI3K-Akt anti-apoptotic and survival pathways, as they were blocked by the PI3K inhibitor, wortmannin [50].

HGF/Met and stem cell therapy for cardiac repair: rationale and in vivo studies

The proposal to combine autologous adult progenitor/stem cell therapy with ex vivo genetic manipulation of these cells might be an innovative approach for the therapeutic delivery of target molecules instead of the standard delivery of plasmid DNA or recombinant proteins. In this context, one possible strategy, among several, would be to combine the regenerative capacity of the HGF/Met signaling pathway with that of CSCs, by creating HGF- or Met-overexpressing CSCs, which are then able to increase the level and duration of HGF and/or Met expression after CSC transplantation in the infarcted myocardium.

Although expressed only at low levels, CSCs already express both HGF and MET [50]. Despite the fact that many growth factors, including HGF, are released in the infarcted myocardium, it is possible that the endogenous levels of the ligand or receptor (HGF and Met) are insufficient to stimulate resident stem cells to take part in regenerating heart tissue, or to protect them from apoptosis. Thus, transplantation of CSCs overexpressing Met in the infarcted myocardium would sensitize the transplanted CSCs to HGF. On the other hand, resident CSCs may exit from quiescence when challenged with high doses of HGF after transplantation of HGF-overexpressing CSCs, and the activated state of CSCs may be maintained by the positive feedback loop created by HGF signaling. Nevertheless, the in vivo use of CSCs for transplantation in infarcted cardiac tissue may be limited by the increased level of effort required to isolate these stem cells compared with other stem cell sources (the adipose tissue, for example). So far, studies employing adult stem cells for HGF gene transfer into the heart have employed the human bone marrow- and adipose tissue-derived stem cells [21, 73, 75]. Overall, these studies indicate the possibility to blunt ischemic injury with transplantation of adult stem cells combined with HGF gene transfer. Zhu and colleagues [75] investigated the effects of endogenous transplantation of human adipose tissue-derived stem cells overexpressing human HGF (hHGF) into a rat model of acute myocardial infarction. The infarction protocol in this study consisted of a 30-min coronary artery occlusion by ligating the left anterior descending coronary artery. Twenty-four hours after the infarction, adipose tissue-derived stem cells (AMI/ADSC group) or adipose tissue-derived stem cells over-expressing HGF (AMI/ADSchHGF group), or phosphate-buffered saline (AMI/PBS group, as control) were injected into the infarcted rats via the vena caudalis (1×10^8 cells in 1 mL of PBS, in the case of cells). The authors demonstrated that cardiac function parameters, including ejection fraction (EF), fractional shortening (FS), left ventricular end-diastolic (LVEDd) and end-systolic diameters (LVESd) were improved at 7, 14, and 28 days after cell transplantation in contrast with the AMI/PBS group. Furthermore, the authors found that transplantation of adipose tissue-derived stem cells induced angiogenesis by suppressing fibrosis and increasing capillary density in the ischemic myocardium. More importantly, compared to the AMI/ADSC group, these effects were enhanced in the AMI/ADSchHGF group. In the study by Guo et al. [21], the authors

investigated the effects of bone marrow-derived MSC overexpressing HGF (MSC-HGF) on post-ischemic heart failure. In this study, 4 weeks after myocardial infarction (induced by coronary artery occlusion by ligation of the left anterior descending coronary artery) in rats, MSC-HGF or MSC or PBS were injected into the infarcted area by multiple intramuscular injections. After 8 weeks from coronary occlusion, the MSC-HGF groups featured better left ventricular systolic and diastolic function compared with the other groups. The authors observed enhanced angiogenesis and reduced apoptosis. Using an innovative approach consisting of the delivery of bone marrow mononuclear cells (BMNC) by an injectable poly-ethylene-glycol-treated (PEGylated) fibrin biomatrix that covalently binds HGF, Zhang et al. [73] showed improved cardiac function in a mouse model of myocardial infarction again after left anterior descending coronary artery ligation. Mice were randomized to receive an intramyocardial injection of saline or an HGF-loaded injectable biomatrix with or without entrapped BMNC. At days 14 and 28 after the infarction, the authors observed an increase in EF and improvements of LVEDd and LVESd, together with lower levels of apoptosis in HGF-treated groups. Finally, Shabbir et al. [58] have recently shown the possibility of facilitating the recruitment of CSCs to the site of myocardial injury by overexpressing various growth factors, including HGF. The study investigated the effects of intramuscularly injected bone marrow MSC or MSC-conditioned medium containing high levels of HGF, vascular endothelial growth factor (VEGF) and IGF into TO2 hamsters, a model of dilated cardiomyopathy harboring a genetic defect in the δ -sarco-glycan gene. Both the MSC and the MSC-conditioned medium groups showed improved ventricular function 1 month after the injection, and this was associated with mobilization of c-kit-positive CPCs.

The majority of clinical trials published to date have used bone marrow-derived mononuclear cells (BMMNC), which include EPC (CD34+, CD133+) and MSC. CSCs, such as MSC, have several features such as the capability to contribute to angiogenesis or vasculogenesis, to home to injured myocardium, and to survive long term after engraftment in newly injured myocardium that make them suitable for cardiovascular cell therapy. However, compared with CSCs, MSC [4] or other types of progenitor cells such as multipotent adult progenitor cells (MAPC) [64] have the advantage that they do not require immunosuppressive therapy and therefore they have been used in allogeneic transplantation. EPCs, on the contrary, have the disadvantage of poor long-term survival after engraftment in infarcted myocardium. Unlike MSC, MAPC and EPCs, CSCs are naturally predisposed to differentiate into all three cardiac cell lineages (endothelial cells, cardiomyocytes and smooth muscle cells). All these features have provided a solid rationale for using CSCs as a vehicle for HGF and/or Met, and justified a move toward clinical applications. The first human study of CSCs “Myocardial Regeneration Using Cardiac Stem Cells (SCIPIO)” (registered at www.clinicaltrials.gov) started in 2009 and is currently in the recruiting phase.

Lentiviral and non-lentiviral vectors to deliver HGF/Met genes into CPCs

The introduction of exogenous genes such as HGF and Met in CSCs might be a useful strategy to specifically tailor CSC for cardiovascular repair. Therefore, it is of utmost importance to develop efficient gene delivery systems that achieve high levels of expression of potentially therapeutic genes in CSCs. Important experimental variables that should be taken into account for a successful gene therapy include multiplicity of infection, length of time or viral incubation and medium used for viral incubation. Optimal assessment of such experimental conditions would increase gene transfer efficiency and obviate the need for selective antibiotic-based enrichment and long-term culture, which may contribute to senescence or compromise their long-term engraftment efficiency and/or multipotency [51]. In addition, by increasing gene transfer efficiency, fewer cells may be required to achieve a therapeutic effect. This justifies the use of lentiviral vectors for transducing adult stem cells, by virtue of their ability to transduce both dividing and non-dividing cells and their relative

ease of use and comparable nature to adeno-associated virus (AAV) which is clinically preferred. Lentivirus-based systems would be ideal vectors for the transduction of adult stem cells since they overcome some problems, such as the short duration of gene expression and the occurrence of significant inflammatory responses, which plague other types of gene vectors (such as adenoviruses) for the transduction of adult stem cells. Lentiviruses are a retrovirus subgroup that includes the human type 1 immunodeficiency virus (HIV). While retroviral systems are inefficient in transducing non-dividing or slowly dividing cells, lentivirus-based vectors, after being pseudotyped with vesicular stomatitis virus glycoprotein G (VSV-G) (i.e., using the glycoprotein envelope from the vesicular stomatitis virus to package recombinant retroviruses) [15], have the capacity to mediate genome integration into both non-dividing and dividing cells (Fig. 3). There is evidence that lentiviral vectors can also transduce more primitive, quiescent progenitors with stable transgene integration [11]. In comparison with retroviral vectors, lentiviral systems allow the immediate transduction without prior expansion, or with growth factor stimulation for only short exposure times. Compared with adenoviral vectors, lentiviral vectors offer also the major advantages of causing little or no disruption of the target cells and of not promoting any inflammatory response [30]. Studies employing lentivirus for HGF gene transfer into human adult stem cell have been limited so far to human bone marrow- and adipose tissue-derived stem cells [21, 75]. AAV vectors represent an alternate type of vector that may also be used for long-term transgene expression in the heart through cell-based therapy [60]. Like lentivirus, AAV can stably integrate into the host genome providing long-term transgene expression, with a minimal inflammatory response. However, AAV can cause insertional mutagenesis and can only carry genes which are less than 5 kb [14].

A possible drawback of the use of lentiviral and AAV vectors for delivering genes that codify growth factors might be that they can cause a chronic overexpression of the protein, with an uncertain therapeutic effect. Short-term gene expression of the growth factor gene would be desirable if the goal is to deliver a secreted protein such as growth factors like IGF-1, vascular endothelial growth factor (VEGF) and HGF, while long-term expression would be preferable if the goal is to express membrane proteins such as receptors for growth factors which require stable expression. Possible strategies to induce short-term gene expression of the transgene include plasmid transfection or adenoviral vectors [48]. Limitations of these strategies are the low transfection efficiency with plasmids and the immunogenic response of the host with adenovirus. We are currently verifying these hypotheses in our laboratory and at the same time testing the appropriate type of vector for gene transfer of HGF and its receptor Met in CSCs.

Perspectives and open questions

The use of CSCs in patients with myocardial infarction and heart failure requires the establishment of a large, expanded bank of CSCs. The use of expanded CSCs has inherent caveats associated with the difficult accessibility, a heterogeneous cell population and the stability of the desired CSC phenotype in *in vitro* culture. However, their capability to home and to survive into injured myocardium, together with their natural predisposition to differentiate into all three cardiac cell lineages, makes these cells attractive for *in vivo* trials. The introduction of therapeutic genes into CSCs by modifying them *ex vivo* (by transfection before implantation) to allow them to express one or more selected genes that might selectively promote resident CSC proliferation, migration, engraftment, and differentiation would represent an interesting additional potential currently being explored to enhance therapeutic effects of transplanted CSCs.

An important question in the context of gene delivery into CSCs relates to the immunogenicity of the transduced cells. Indeed, one of the major barriers to stable gene

transfer by lentiviruses is the development of innate and adaptive immune responses to the delivery vector, even less than for adenoviral vectors, and the transferred therapeutic transgene (i.e., HGF/SF and Met). However, several strategies can be used to overcome the host immune response to the transfer vector, including the injection of vector doses below the threshold that determines an increased inflammatory gene expression or the recruitment of inflammatory cells [16, 33]. Therefore, the only immunologically relevant protein here would remain the HGF or Met proteins. Recent studies have shown the role of HGF in promoting immune-mediated disorders. HGF promotes B- and T-lymphocyte migration [3, 65, 68], counteracts the immunosuppressive effects of tumor growth factor (TGF)- α , a potent immunosuppressive cytokine [28, 40, 61], suppresses dendritic cell function [45] and stimulates mast cells in response to *Listeria* infection [37]. However, other studies conducted in the mouse model of allogeneic heart transplantation have shown that HGF reduces acute and chronic rejection of the allograft, indicating that HGF might induce allograft tolerance and reduce acute and chronic rejection [71]. Thus, the role of HGF and Met in immunity is not well defined and whether Met-transduced CSCs can trigger Met-specific immune response in vivo after transplantation remains to be investigated.

Conclusions

CSCs can be genetically manipulated ex vivo with the purpose of reintroducing them in vivo, where they could provide therapeutically relevant levels of HGF and Met. The high probability that this therapy may be long-lasting represents a substantial theoretical advantage over treatments with recombinant proteins. In addition, the expanded population of autologous CSCs can be reliably stored for future repeated use, if and when needed. Therefore, expanded CSCs can be used to provide a source of autologous cells for the expression and secretion of any of a wide variety of proteins that might facilitate local intra-cardiac, regenerative effects.

Acknowledgments

The original studies here reported were supported by National Institutes of Health grants R01 HL55757, HL-70897, HL-76794, and HL78825 (to R. Bolli); and grants by the Italian Ministry of Research and Scientific Research and the Istituto Italiano Ricerche Cardiovascolari (to Prof. R. De Caterina).

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Fig. 1.

The structure of hepatocyte growth factor/scatter factor (HGF/SF) (**a**) and Met tyrosine kinase receptor (**b**). HGF consists of six domains: an amino-terminal domain (N), four kringle domains (K1–K4) and a serine proteinase homology (SPH) domain. The mature form of Met consists of an extracellular α -chain and a longer β -chain. The β -chain encompasses the remainder of the Met ectodomain, the transmembrane helix and the cytoplasmic portion. The latter contains the juxtamembrane and kinase domains as well as a carboxy-terminal tail that is essential for downstream signaling. *S* semaphorin domain, *C* cysteine-rich domain, *Ig* immunoglobulin domain, *K* kinase domain, α and β refer to the subunits of the receptor that are present after proteolytic cleavage

Fig. 2. Signaling pathway of HGF and Met. *PI3-K* phosphatidylinositol(PI)3-kinase, *MEK* MAPK (mitogen-activated protein kinase)/ERK (extracellular receptor kinase)-kinase, *shc* src homology-2 (SH2) and collagen homology; *PKB* Akt or protein kinase B; *Grb* growth factor receptor-bound protein

Fig. 3.

Schematic representation of the transducing vector construct (**a**), packaging construct (**b, c**) and VSV-G Env expressing construct (**d**). *Amp* ampicillin resistance gene, $\Delta U3$ deleted region of the 3' LTR, which allows for biosafety of the vector; *Gag, pol, env* genes coding for envelope proteins, *HGF* gene construct encoding HGF, *Met* gene construct encoding the Met receptor, P_{CMV} cytomegalovirus promoter, 3'-LTR 3'-long terminal repeats for viral packaging, P_{RSV} Rous Sarcoma Virus (RSV) enhancer/promoter, 5'-LTR 5'-long terminal repeats for viral packaging, Ψ psi packaging sequence for viral packaging, *pA* polyadenylation signal, *RRE* HIV-1 Rev response element, *VSV-G* glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) as a pseudotyping envelope. **e** Photographs representing the experimental steps of production of the lentiviral stock (containing the packaged expression construct) by cotransfecting the packaging construct (**b, c**), the VSV-G Env expressing construct (**d**) and transducing vector construct (**a**) into the 293FT virus producer cell line. The expression of the VSV-G glycoprotein causes 293FT cells to fuse, resulting in the appearance of large, multinucleated cells known as syncytia