Stem cell-mediated regeneration of the infarcted heart

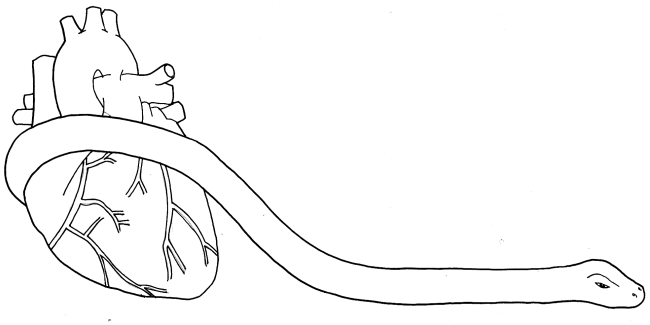
Velde, Susanne van der

Signaling factors in stem cell-mediated repair of the infarcted myocardium

Vandervelde S[1], van Luyn M.J.A.[1], Tio R.A.[2], and Harmsen M.C.[1]

Departments of Pathology & Laboratory Medicine[1] and Cardiology[2], University of Groningen, Hanzeplein 1, NL 9713 GZ Groningen, the Netherlands

Journal of Molecular and Cellular Cardiology 39 (2005) 363-376



Abstract

Myocardial infarction leads to scar formation and subsequent reduced cardiac performance. The ultimate therapy after myocardial infarction would pursue stem cell-based regeneration. The aim of stem cell-mediated cardiac repair embodies restoration of cardiac function by regeneration of healthy myocardial tissue, which is accomplished by neo-angiogenesis and cardiogenesis.

A major reservoir of adult autologous stem cells distal from the heart is the bone marrow. Adequate regulation of signaling between the bone marrow, the peripheral circulation and the infarcted myocardium is important in orchestrating the process of mobilization, homing, incorporation, survival, proliferation and differentiation of stem cells, that leads to myocardial regeneration.

In this review, we discuss key signaling factors, including cytokines, chemokines and growth factors, which are involved in orchestrating the stem cell driven repair process. We focus on signaling factors known for their mobilizing and chemotactic abilities (SDF-1, G-CSF, SCF , IL-8, VEGF), signaling factors that are expressed after myocardial infarction involved in the patho-physiological healing process (TNF-α, IL-8, IL-10, HIF-1α, VEGF, G-CSF) and signaling factors that are involved in cardiogenesis and neo-angiogenesis (VEGF, EPO, TGF- β, HGF, HIF-1α, IL-8).

The future therapeutic application and capacity of secreted factors to modulate tissue repair after myocardial infarction relies on the intrinsic potency of factors and on the optimal localization and timing of a combination of signaling factors to stimulate stem cells in their niche to regenerate the infarcted heart.

Introduction

Myocardial infarction leads to adverse remodeling that depresses cardiac function. The cardiomyocytes that survive ischemia primarily respond with cellular hypertrophy rather than proliferation, due to the limited mitotic capacity of adult cardiomyocytes. Under physiological circumstances this limited mitotic capacity restricts the repair of the ischemic myocardium leading to replacement by fibrotic tissue, which disrupts proper contractile function resulting in decreased cardiac performance.

One way to intervene in this downward spiral and thereby repair the myocardium is to replace fibrotic tissue by healthy myocardial tissue consisting of cardiomyocytes and vasculature that forms a syncytium with the spared myocardium. A source for generation of myocardial cells is formed by stem cells. Stem cells are defined as cells capable of self renewal and differentiation into various cell types with specialized structure and function. Stem cells are regarded as a new opportunity to intervene in degenerative disease of liver, brain and heart [1-3]. Although adult stem cells are present in several mature tissues e.g. muscle, brain, skin and liver, in this review we focus on bone marrow-derived stem cells (BMSC) and cardiac stem cells (CSC).

The ultimate goal of stem cell-mediated cardiac repair is regeneration of healthy, functionally integrated, myocardial tissue. To date, three distinct (experimental) treatment modalities of myocardial infarction involving stem cells can be recognized: 1) stem cell transplantation: adult stem cells can be harvested and injected (locally) into the infarcted recipient; 2) stem cell mobilization: availability of stem cells for cardiac repair can be augmented by enhancing mobilization of stem cells from the bone marrow; and 3) manipulation by local factors: stem cells (BMSC and CSC) can be manipulated by altered expression of cytokines and growth factors to improve their local reparative capacity in the infarcted myocardium. It is conceivable that the optimal stem cell-mediated repair will be a combination of different modalities.

With the different modalities of stem cell therapy, three recurrent substantial components can be recognized: the bone marrow as major reservoir of stem cells, the infarcted myocardium as place of repair and the peripheral circulation as transport way of the stem cells and signaling factors. Signaling among these different components is essential for regulation, but can also be regarded as a target to enhance stem-cell mediated repair. In this review, we discuss signaling factors involved in stem cell mobilization from the bone marrow, in directing and engrafting stem cells to the ischemic lesion in the heart and factors involved in differentiation and proliferation of cells pivotal for healthy myocardium.

Stem cell transplantation studies

The first stem cell-mediated treatment modality after myocardial infarction we mentioned here was stem cell transplantation i.e. harvesting stem cells from the bone marrow or the peripheral blood and transplanting them into the infarcted recipient.

Numerous studies of BM stem cell transplantation in infarcted myocardium have been published over the past few years. Many of them claim improved cardiac function and attenuation of adverse remodeling. Since these studies have been reviewed extensively, we will not enlist them here [4-6]. The methods used in stem cell transplantation studies are varying. Different populations of stem cells were used in various numbers at different time points after ischemia in different infarction models in several mammalian species including man [4]. Notwithstanding the divergent methods used, some reports claim differentiation of transplanted stem cells into cardiomyocytes. However, there are also

recent publications stating quite the contrary: differentiation of stem cells is an extremely rare event [7] and could be potentially due to cell fusion [8,9]. Two recent reports, in which sophisticated methods to prove differentiation were used, claimed failure to replicate findings of differentiation of transplanted stem cells into cells other than hematopoietic cells [9,10]. Thus, whether bone marrow-derived stem cells can become cardiomyocytes after transplantation is still a matter of debate.
Also, the mechanism of functional improvement after stem cell transplantation pleads for more thorough investigation. For instance, how do the transplanted cells improve cardiac function if they do not abundantly differentiate into cardiomyocytes? Is enhancing perfusion by stem cells that differentiate into vasculature already sufficient to improve function and will this improvement last? Or could the function of BMSC be orchestration of cardiac repair rather then actual structural incorporation and differentiation?
The reported stem cell transplantation studies were the first step in attainable regenerative therapy after myocardial infarction that opened a plethora of novel stem cell-based treatment modalities. However, the time has come to refine stem cell transplantation by taking a closer look at stem cell function and their environmental needs. Besides finding (and isolating) the most suitable stem cell subpopulation in sufficient quantities, we have to focus on the optimal time window and best localization for incorporation and differentiation of stem cells. This time window depends on the patho-physiological (inflammatory) process following myocardial infarction. To be able to create the ideal recipient environment, knowledge of factors that are involved in BMSC signaling for mobilization, homing, incorporation, survival, differentiation and proliferation is invaluable.

Bone marrow as stem cell reservoir
Bone marrow can be regarded as the major reservoir of stem cells. Upon proper stimulation, stem cells can be activated and subsequently mobilized into the peripheral blood. The niche, in which bone marrow stem cells remain quiescent, is comprised of a diverse population of stromal cells and extracellular matrix components, such as fibronectin, collagens and proteoglycans [11]. Interactions of stem cells with the niche and release of anchored stem cells and subsequent trafficking from the bone marrow into peripheral blood is under thorough investigation. Only recently, surface molecules on BMSCs, like very late antigen 4 (VLA-4) [12], glycosaminoglycan hyaluronan receptor CD44 [13] and Selectins [14], that play a role in adhesive interactions of BMSCs in the bone marrow have been recognized. Understanding mechanisms of stem cells mobilization is critical in designing new strategies for enhancement, but reaches beyond the scope of this review.
Moreover, the actual constitution of the most pluripotent stem cell or stem cells committed to specific organs that resides in the bone marrow is not known yet. Ratajczak and co-workers hypothesize that the bone marrow not only harbors hematopoietic stem cells, but also provides a 'hideout' for circulating tissue-committed stem cells of various organs (muscle, liver, brain, heart)[15,16]. During stress or tissue injury (for example myocardial infarction) the levels of the tissue-committed stem cells in the peripheral blood are increased and available for damage repair [15,16].

Nevertheless, a wide number of factors are known to increase the mobilization of bone marrow-derived stem cells into the peripheral blood, among which are the factors G-CSF, GM-CSF, SCF, VEGF, IL-8 and SDF-1, which we chose to discuss in this review [17].

Cardiac Stem Cells

Besides exogenous stem cell transplantation, reports emerge that claim the existence of cardiac stem cells. The dogma of the heart as a terminally differentiated organ was challenged by the observation of early cardiac cells in a mitotic state. Beltrami et al. showed proliferation of cardiomyocytes in patients who died 4 to 12 days after myocardial infarction. They observed a ratio of cells undergoing mitosis to the number of cells not undergoing mitosis of 0.08 % in the zone adjacent to the infarct and 0.03 % in the zones distant to the infarcts [18]. Only a few years later the same group of Anversa reported the existence of $Lin^{-}c\text{-}kit^{+}$ cells with properties of stem cells, found in clusters of an (average) density of 0.01% in the rat adult myocardium. A small percentage (7-10%) of these cells showed expression of early cardiac transcription factors, and in vitro experiments with these cells indicate that the isolated $Lin^{-}c\text{-}kit^{+}$ cells are self-renewing, replicate unlimited and could give rise to the main myocardial cell types: cardiomyocytes, smooth muscle cells and endothelial cells i.e. these cells clearly possess stem cell features. The cultured cells were injected into infarcted myocardium of rats. Twenty days after infarction they showed that these cardiac stem cells could contribute to functional cardiac repair [19]. The stem cell transplantation resulted in a band of proliferating regenerating myocardium that reduced the infarct size and was composed of cardiomyocytes and functional blood vessels resembling the neonatal heart [19].

Moreover, notwithstanding the origin of cardiac stem cells, proliferating host cells with early cardiac markers (MEF2 and GATA-4) were identified in sex mismatched heart transplants, a female heart into a male recipient. [20]. However, the ability of these proliferating early host cardiomyocytes to repair the heart after myocardial infarction is insufficient, as can be concluded from a clinical study on sex-mismatched heart transplantation of patients who developed myocardial infarction after heart transplantation. In this study an increase in Y-chromosome positive cardiomyocytes compared to non-infarcted controls was not observed [21].

The cardiac origin of the Y-positive cells found in transplanted hearts is challenged by a study of a small number of sex mismatched bone marrow transplantation patients. Y-chromosome positive cardiomyocytes (0.23%) were found in the hearts, suggesting that these cardiomyocytes originate from the bone marrow [22]. More evidence that a small percentage of cardiomyocytes in the heart may originate from bone marrow-derived cells is provided by animal studies in which bone marrow reconstitution with genetically marked bone marrow cells (GFP, LacZ) was followed by myocardial infarction. In these animals marked cardiomyocytes were detected in the heart after myocardial infarction [23,24].

To summarize, there is evidence for the existence of cardiac stem cells. They are small cells that are present in the myocardium, that can proliferate and have regenerative capacity. They are present in small quantities, but are, under patho-physiological circumstances, incapable of functional cardiac repair after myocardial infarction. It is conceivable, that these cells serve a function in "normal" tissue turnover and might not be equipped to repair severe myocardial damage. Nevertheless, adequate exogenous activation signals given to cardiac stem cells might aid myocardial regeneration.

The infarcted myocardium

The infarcted myocardium forms the recipient environment for stem cells in stem cell-mediated repair. It can also be regarded as target environment for signaling factors.

Knowledge of the physiological healing process and of cytokines and growth factors involved following myocardial infarction is essential for timing and localization of stem cell-mediated repair.

The patho-physiological remodeling process after myocardial infarction can be divided into four phases: 1. Cardiomyocyte death from apoptosis and necrosis, 2. Inflammation characterized by influx of inflammatory cells (primarily macrophages, neutrophils and mestcells) and degradation of extracellular matrix (ECM), 3. Formation of granulation tissue comprised of neovasculature, macrophages and myofibroblasts, and 4. Scar formation [25]. In the inflammatory phase and granulation forming phase, e.g. the early phase after myocardial infarction, there is an abundant upregulation of cytokines and growth factors.

Ignoring differences in species used and differences in myocardial infarction-model, the inflammatory response at the tissue level, starts with a rapid transient increase in neutrophils in the infarcted area. This is followed shortly by an influx of macrophages and not long thereafter a transient accumulation of myofibroblasts [26]. Complement activation has an important role in the initiation of neutrophil, and subsequent monocyte recruitment towards the ischemic myocardium [27,28]. However, after a prolonged period the effect of complement activation wanes and monocyte chemotactic activity becomes more attributable to factors such as TGF-β1 and MCP-1 [27]. Also, free radicals such as Reactive Oxygen Species (ROS), which are formed immediately after ischemia, can directly harm cardiomyocytes and endothelial cells by inducing apoptosis. ROS are also involved in triggering the inflammatory cascade through induction of cytokines [29-31].

The infiltrated inflammatory cells themselves are rich sources of cytokines and growth factors that play a role in the cardiac remodeling process. Inflammation after myocardial infarction is a complex process invoked by cell death, consisting of cellular infiltration and extracellular remodeling, that is orchestrated by cytokines and growth factors. Cytokines and growth factors have different effects dependent on temporal and spatial variability. The complexity of unraveling chronological importance and roles of cytokines lies within their properties of redundancy and pleiotrophy, but also in their synergistic and antagonistic activities [32]. The factors that govern the inflammatory process also dictate the behavior of stem cells in cardiac repair process.

The pro-inflammatory cytokine cascade constitutes the release of TNF-α, IL-1β and IL-6 and is instrumental in the induction of cellular infiltration. It has been suggested that secretion of preformed mast cell-derived TNF-α is essential in upregulating IL-6 in infiltrating leukocytes after myocardial ischemia [33]. These three pro-inflammatory cytokines are not only associated with the orchestration of the inflammatory response after myocardial ischemia, but are also involved in aspects of heart failure itself, for instance left ventricular dysfunction, pulmonary edema, LV remodeling and cardiomyopahty [34-37]. Furthermore, IL-8 also regulates neutrophil recruitment [38,39]. IL-10 inhibits the production of IL-1β, TNF-α, IL-6 and IL-8 and therefore suppresses the inflam matory response and helps to maintain a balance [40].

Hence, in this review we highlight the role of TNF-α (as exemplary pro-inflammatory cytokine) and IL-8 in stem cell-mediated cardiac repair, since these factors are expressed in the infarct after myocardial infarction and therefore may interfere in stem cell engraftment.

A special part of the natural healing process after myocardial infarction is the angiogenicresponse. Two possible sources of endothelialization have been identified: 1) sprouting or endothelial migration from adjacent pre-existing blood vessels [41] or 2) neo-angiogenesis by differentiation from migrated, circulating bone marrow-derived

endothelial progenitor cells (EPCs), i.e. a subpopulation of BMSCs [42]. Thus, restoration of vessel infrastructure is not only important for transport and survival of transplanted or recruited stem cells, but stem cells themselves also appear to play a role in neo-angiogenesis. Evidence for this phenomenon originates from studies that demonstrate that genetically marked bone marrow-derived stem cells were recruited to ischemic limbs of mice, incorporated at the site of neovascularization and accelerated revascularization [43,44]. Notwithstanding whether these BMSC actually differentiate into endothelial cells [45], the fact that stem cells home to ischemic areas to promote neovascularization [44] opens another door for stem cell-mediated cardiac repair. Therefore, in this review we are interested in regulatory factors that promote angiogenesis, such as VEGF, HIF-1α and IL-8.

Signaling factors for stem cell-mediated repair

In stem-cell mediated cardiac repair, three components can be distinguished: 1) the bone marrow as the major reservoir of stem cells, 2) the ischemic myocardial tissue as the place of repair and 3) the peripheral circulation as transport way of the stem cells and signaling factors. Interactive signaling between these components is important for the orchestration of mobilization, incorporation, survival, proliferation and differentiation of stem cells (fig. 1).

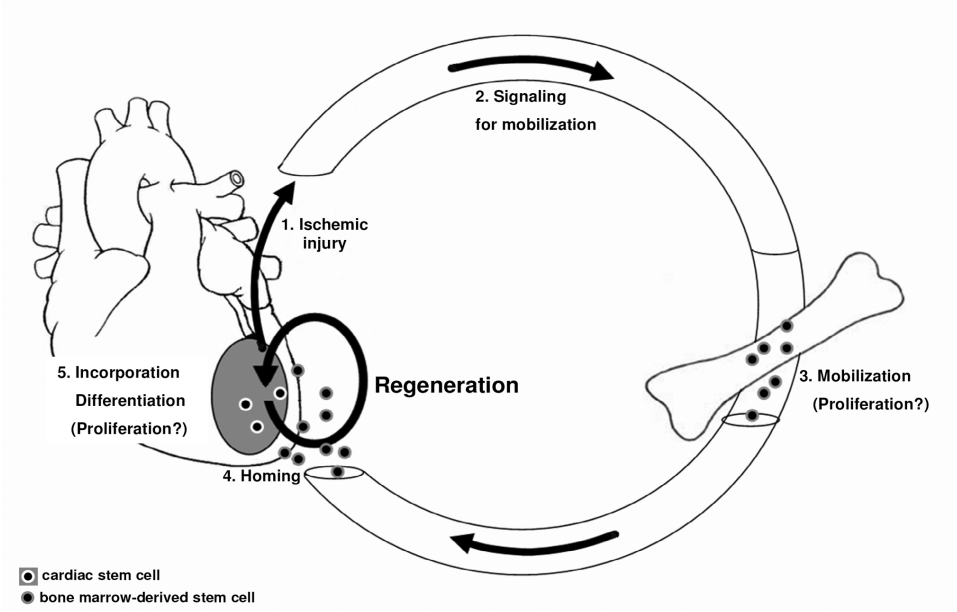


Figure 1. *The signaling between the three fundamental components of stem cell-mediated cardiac repair, i.e. the myocardial infarction, the peripheral circulation and the bone marrow, to acquire regeneration is driven by the substantial processes of mobilization, homing, incorporation, survival, proliferation and differentiation. Whether the mobilization/ homing part can be circumvented by encouraging endogenous cardiac stem cells to support regeneration is under investigation.*

We further discuss the factors that have a significant role in the signaling process. Factors that are important for mobilization include G-CSF, GM-CSF, SCF, SDF-1, IL-8 and VEGF. Factors that are expressed after myocardial infarction and are involved in the subsequent inflammatory process include TNF-α, IL-8, IL-10, HIF-1α, VEGF and HGF. Factors that are potentially involved in the differentiation, proliferation and survival process of stem cells include EPO, TGF-β family, VEGF and HGF. We have highlighted only a selection of key signaling factors, because these factors are exemplary in stem cell-mediated repair.

A. TNF-α

Tumor Necrosis Factor-alpha (TNF-α) is a very potent pro-inflammatory cytokine that is rapidly secreted after ischemic injury. This pro-inflammatory cytokine is exemplary among the pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6. TNF-α is produced by monocytes/macrophages [46], degranulating mast cells [33], cardiac fibroblasts [47] and cardiomyocytes [48].

Data on effects of TNF-α on the heart are complex and often are contradicting. Although TNF-α overexpression leads to a phenotype of heart failure [49,50] and TNF-α seems to have an early role in increase of infarct size [51-54], these negative effects of TNF-α after myocardial infarction could not be confirmed clinically by anti-TNF therapy [55,56]. Moreover, TFN-α induces resistance of cardiomyocytes to hypoxic stress in vitro [57,58].

Similarly, the role of TNF-α in stem cell mobilization is also comples and variable. In vitro, migration of embryonic stem cells was enhanced by neonatal rat cardiomyocytes overexpressing TNF-α, which was attenuated after pre-incubation of the embryonic stem cells with antibody against TNF-RII, which suggests a chemoattractive response of stem cells towards TNF-α [59]. However, although a chemoattractive response was seen with embryonic stem cells, proliferation of BMSCs was inhibited when TNF-α was added to an in vitro single cell proliferation assay. Moreover, the number of BMSCs was increased in TNF-receptor p55 deficient mice [60]. This is in concordance with stem cells numbers observed in patients with heart failure, in which CD34+ stem cells and endothelial progenitor cells were inversely related to TNF-α titers in serum. Circulating stem cell numbers were decreased where TNF-α levels were increased in patients with advanced stage of heart failure, which could be related to a myelosuppressive role of TNF-α [61].

To conclude, TNF-α is a pleiotrophic cytokine that works in a temporal and spatial dependent manner. Early blocking of TNF-α, within the first hours or days after MI, could decrease infarct size. Moreover, TNF-α is shown to have chemoattractive features, but also has anti-proliferative features on BMSCs. To conclude, usage of TNF-α in stem cell mediated cardiac repair can be invaluable if timed and located correctly, but can be deleterious, if not.

B. Interleukin-8

The CXC chemokine Interleukin-8 (IL-8/CXCL8) is expressed by various cell types, such as monocytes and endothelial cells, and is strongly upregulated by pro-inflammatory cytokines [62]. IL-8 is a chemoattractant and activator of neutrophils. Overexpression of IL-8 *in vitro* increases the adhesion of neutrophils to isolated cardiomyocytes *in vitro*, which induces cardiomyocyte death [63]. IL-8 mRNA is markedly increased after coronary occlusion followed by reperfusion, although without reperfusion only minimal amounts can be detected [63].

Besides its inflammatory effect, Il-8 is also able to rapidly induce stem cell mobilization, albeit at lower numbers as compared to G-CSF stimulation and also for a shorter time

period [64,65]. Nevertheless, in G-CSF treated patients the level of IL-8 positively correlated with BMSC (CD34+) numbers before and during treatment, which suggests that IL-8 production may be of importance in G-CSF induced stem cell mobilization [66]. The short mobilizing time period of IL-8 was demonstrated in a study with primates, in which a single injection of human recombinant IL-8 resulted in a 10- to 100 fold increase in numbers of circulating hematopoietic progenitor cells in the peripheral blood, which returned to almost pretreatment values within four hours after IL-8 injection [67].
In IL-8 induced mobilization, the enzyme matrix metalloproteinase-9 (MMP-9) is of importance, since pretreatment with an anti-MMP-9 antibody in primates prevents BMSC mobilization by IL-8 [68]. Neutrophils release MMPs that are able to cleave extracellular matrix molecules such as Kit-ligand inside the bone marrow and thereby support release of BMSCs into the peripheral blood. This coherence between neutrophils, MMPs and IL-8-induced mobilization is affirmed by the observation of a reduction in IL-8 induced mobilization of BMSCs in absence of neutrophils [69]. Thus, in absence of either neutrophils or MMP-9, IL-8 induced BMSC mobilization is reduced. Although neutrophils and IL-8 seem to play a role in BMSC mobilization, treatment with anti-neutrophil monoclonal antibody lowered the levels of IL-8, but also decreased infarct size in rats [70]. This suggests that neutrophils have a negative effect on infarct size, but stimulate the production of IL-8. Whether the positive effect of IL-8 and neutrophils on mobilization outweighs the negative inflammatory effect in myocardial infarction and on infarct size, can be doubted.

Nevertheless, a space and time dependent introduction of IL-8 can be interesting to enhance its positive effects. Since serum levels of IL-8 have a positive effect on rapid early mobilization of BMSCs and since IL-8 may augment the local adverse response in the heart after myocardial infarction, early blockade of the effects of IL-8 in the heart and overall at later time points can be interesting for stem cell mediated cardiac repair.

D. G-CSF and SCF

Granulocyte and Granulocyte/Macrophage Colony-Stimulating Factor (G-CSF and GM-CSF) and Stem Cell Factor (SCF) are hematopoietic factors, that are involved in proliferation, differentiation and survival of bone marrow derived stem and progenitor cells [71-73]. SCF, also known as c-kit Ligand or Steel Factor, binds to c-Kit, a receptor expressed on the surface of stem and progenitor cells and has a chemoattractant effect on these cells [74]. Although mRNA of M-CSF and SCF is abundantly expressed in the normal heart, it is actually downregulated after permanent coronary artery ligation in mice. G-CSF mRNA expression was not detected in the heart at all and GM-CSF mRNA expression in the normal heart was negligible [75,76].
G-CSF and GM-CSF are used clinically to increase the rate of recovery of hematopoietic cells after bone marrow transplantation. In rodents, the addition of SCF to G-CSF increased the levels of proliferation in the bone marrow prior to mobilization [77]. The group of Orlic et al. was one of the first to show that combined treatment of G-CSF and SCF, given from 5 days prior to 3 days after coronary artery ligation in mice, attenuates adverse cardiac remodeling 27 days after myocardial infarction, such as decreased infarct size, less ventricular dilatation and decreased diastolic stress. Moreover, they observed proliferation of cardiomyocytes [78]. This observation was questioned by other groups that failed to demonstrate cardiomyocyte proliferation as a consequence of G-CSF and SCF stimulation. In a non-human primate model, after a single administration of G-CSF and SCF four

hours after coronary ligation, an increase in myocardial blood flow and endothelial cell differentiation was observed, although no differentiation into cardiomyocytes was seen [79]. Also, in a study in which 8 weeks after permanent ligation of the coronary artery in rat G-CSF was administered for 5 days, no induction of cell proliferation or improvement of cardiac function was observed [80].
G-CSF administration for 5 days starting directly after coronary artery ligation in mice also resulted in improved cardiac function, less remodeling and increased number of bone marrow-derived capillaries, but again not cardiomyocyte proliferation [81]. Altogether, this data suggest a time and dose dependent effect of G-CSF (in combination with SCF) administration, which can result in improvement of cardiac function and potentially even in cardiomyocyte proliferation, although this is only observed by some authors.
An increase in influx and maturation of inflammatory cells is detrimental in the absence of a proper regulatory mechanism, and therefore forms a conceivable disadvantage of G-CSF and SCF treatment. SCF is induced in and secreted by infiltrating macrophages in the ischemic myocardium and attracts mast cell precursors [82]. G-CSF and GM-CSF are not only associated with stem cell mobilization, but they are also known to stimulate the development of committed progenitor cells into traditional hematopoietic cells, mainly granulocytes and macrophages [83]. Whether the increase of neutrophils and macrophages would be a negative effect of G-CSF is contradicted in a study that aimed at defining the modifying effect of G-CSF on the healing process following MI [84]. In this study an increase of macrophages and neutrophils was found 7 days post-MI, after five days of treatment with G-CSF, which was related to the enhanced absorbtion of necrotic tissue and coincided with an enhanced induction of regenerating myocardial cells and improvement of cardiac function [84].
After promising results of G-CSF therapy in rodents, the safety and efficacy of G-CSF therapy was tested clinically. Although results of the first clinical study in which G-CSF alone or in combination with intracoronary infusion of collected peripheral blood stem cells was given to myocardial infarction patients who underwent stenting of the coronary artery were promising, they also found a higher incidence of in-stent restenosis [85]. This hazardous side-effect was not observed in a study with more patients [86]. In two other clinical trials (FIRSTLINE AMI and STEMI) with patients undergoing percutanous coronary intervention, show improvement of cardiac function after G-CSF therapy [87-89]. Contradictorily, others found that the functional activity of mobilized Hematopoietic Stem Cells (HSCs) of patients with chronic ischemic heart disease, measured as the migratory response towards SDF-1 and VEGF-A, was markedly reduced after G-CSF-induced mobilization [90]. Potentially, this paradox is related to the origin of BMSC source, as can be concluded from a study in which the origin of G-CSF mobilized cells that repair infarcted myocardium is investigated [91]. In this study it is suggested that clonally purified nonhematopoietic mesenchymal stem cells, rather than HSCs form the origin of BM-derived cardiomyocytes after G-CSF therapy [91]. Nevertheless, CSFs and SCF are interesting factors for stem cell mediated cardiac repair, since these have the ability to mobilize stem cells from the bone marrow and apparently have an attenuating effect on cardiac remodeling. The efficacy of stem cell incorporation and thereby of stem cell-mediated repair could be enhanced by upregulation of SCF in the infarcted area. SCF is downregulated in the heart after myocardial infarction under physiological circumstances, which is unfavorable for homing of BMSCs to the damaged heart. Also, upregulated CSFs in the ischemic heart could enhance local proliferation, differentiation and survival of BMSCs.

To conclude, elevated levels of both SCF and CSFs in the circulation would enhance mobilization of BMSC from the bone marrow. Upregulation of both SCF and CSFs in the heart after myocardial infarction would potentially foresee in the incorporation, proliferation, differentiation and survival of BMSC in the infarcted heart, which are essential steps in stem cell-mediated repair.

E. SDF-1 and its receptor CXCR4

Stromal Derived Factor-1 (SDF-1) and its receptor CXCR4, are crucial in stem cell mobilization. In vitro assays showed that migration of BMSC (cultured CD34+ cells) towards SDF-1 is strong and dose-dependent [92]. Moreover, intravenous injection into mice of an adenoviral vector encoding SDF-1α resulted in increased mobilization of hematopoeitic stem cells [93,94]. Mobilization of stem cells is inhibited by neutralizing antibodies towards CXCR4 and SDF-1 [95]. Also, overexpression of CXCR4 by a lentiviral gene transfer on human BMSC (CD34+) improved migration towards lower SDF-1 levels and improved survival of these CXCR4 overexpressing progenitor cells [96]. The SDF-1 – CXCR4 axis is involved in the chemoattraction of BM-derived cardiac progenitor cells after myocardial infarction [15].

The suggested mechanism of stem cell mobilization from the bone marrow by SDF-1 is that SDF-1 induces upregulation of metalloproteinase-9 (MMP-9) activities, which cause shedding of soluble Kit-ligand (SCF) and thereby liberate c-Kit positive stem cells into the circulation [97]. Nevertheless, MMP-9 knockout mice did not show a disturbed BMSC mobilization [98], suggesting that other factors also contribute to the mobilization mechanism.

Beside their effect on stem cell mobilization, SDF-1 and CXCR4 are also important in cardiogenesis and vasculogenesis. Both SDF-1 deficient mice and CXCR4 deficient mice die perinatally and have defects in cardiac ventricular septal formation, bone marrow hematopoiesis and organ-specific vasculogenesis [99,100]. In the normal adult heart, SDF-1 is expressed constitutively, and was shown to be upregulated after myocardial infarction in rats [80,101].

Askari et al. re-upregulated SDF-1 expression 8 weeks after myocardial infarction by intramyocardial transplantation of stably transfected cardiac fibroblasts overexpressing SDF-1 in combination with G-CSF therapy. This was associated with much greater numbers of BMSCs (c-Kit or CD34 positive) and endothelial cells in the heart and resulted in an increase of vascular density and improvement of left ventricular function. Strikingly, this improvement of function was not related to proliferation of cardiomyocytes [80]. This implies again that improvement of left ventricular function can be achieved by improved cardiac perfusion alone.

A hurdle in the clinical usage of SDF-1 as single therapy could be a reduced migratory response towards SDF-1 of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease, despite similar number of cells [102]. Age-related reduction of migration towards SDF-1 was also demonstrated in mice, in which optimal migration was seen with BMSCs of 1 month- old mice, which was greatly reduced with BMSCs of 2 month-old mice [15].

To conclude, SDF-1 and its receptor CXCR4 are important in orchestrating mobilization of stem cells from the bone marrow and migration of BMSCs to the ischemic myocardium. They also have a substantial role in embryonic cardiogenesis, which, to date, is not affirmed in later developmental stages. Moreover, SDF-1 and its receptor contribute substantially

to both embryonic vasculogenesis as well as to revascularization of the infarcted myocardium. Taken together, upregulation of both factors is crucial in stem cell-mediated repair.

F. HGF

Hepatocyte Growth Factor (HGF) was, as the name suggests, originally associated with liver regeneration, but was rediscovered as a growth factor affecting various tissues and cell types. Activities of HGF include induction of cell proliferation, cell motility and dissociation, morphogenesis as well as inhibition of cell growth. Upon activation HGF is expressed by fibroblasts, smooth muscle cells, mast cells, macrophages, endothelial cells and leukocytes (see for review: [103]). Expression of HGF is induced by mediators such as IL-1, PDGF, bFGF and G-CSF and is suppressed by TGF and glucocorticoids [104,105].

HGF and its receptor are also involved in cardiogenesis, in which it is transiently expressed during early cardiac development [106]. The levels of HGF mRNA and of its receptor (c-met) are normally low in the heart, but are upregulated for at least fourteen days after permanent coronary artery occlusion in rats [107]. Both in vitro and in vivo, HGF enhanced survival of cardiomyocytes under ischemic conditions [107,108]. Moreover, intramyocardial gene therapy with HGF after myocardial infarction resulted in increased angiogenesis and preserved cardiac contractile function [109-111].

The role of HGF in stem cell-mediated repair is probably not only locally in the heart, but also stretches to the bone marrow, where HGF is involved in adhesion of stem cells to their bone marrow microenvironment. HGF is produced by bone marrow stromal cells and it promotes adhesion, proliferation and survival of hematopoietic stem cells [112]. Fujii et al. showed an inverse correlation between serum levels of HGF and the number of BMSC (CD34+) mobilized cells in G-CSF treated patients, which could be explained by the encouragement of BMSC adhesion to the bone marrow microenvironment orchestrated by HGF [105]

The mechanism of HGF in stem cell mediated repair in the myocardium lies furthermore in its ability to create an adhesive microenvironment in the heart after stem cells are recruited there. This is demonstrated in a study of HGF transfected BMSCs transplanted in infarcted myocardium [113]. Bone marrow-derived mesenchymal stem cells transfected with HGF that were intramyocardially injected in the borderzone of permanently ligated rat hearts incorporated in the heart, which resulted in a reduced infarct size, increased number of capillaries, as well as reduced collagen content and improved cardiac function four weeks after transplantation. The authors also claim that the incorporated mesenchymal stem cells were morphologically indistinguishable from the surrounding cardiomyocytes, although their proof of differentiation of BMSCs was only based on morphological grounds [113].

In conclusion, HGF seems to be a promising factor in stem cell mediated cardiac repair. HGF increases survival of cardiomyocytes after oxidative stress, and thereby reduces apoptosis. When overexpressed in the heart, HGF increases angiogenesis and improves the function of the infarcted heart. Furthermore, HGF is involved in anchoring stem cells to a microenvironment, e.g. the bone marrow and the ischemic myocardium, where HGF promotes adhesion, survival and proliferation of the BMSCs.

G. HIF

Hypoxia-inducible factors (HIF) are early transcriptional regulators of the response to hypoxia, which activate pathways that increase oxygen delivery and promote adaptive pro-survival responses. Among the many target genes of HIF are erythropoeitin (EPO),

endothelin and VEGF (with its receptor Flk-1) [114,115]. Episodes of intermittent hypoxia in wildtype mice induce HIF-1α quantities sufficient to induce EPO production in the kidneys, which did not occur in HIF-1α nullizygous mice. EPO production is associated with cardiac protection after ischemia-reperfusion injury [116].
HIF-1α is essential in normal cardiac development during embryogenesis. Complete HIF-1α deficiency in mice results in lethal cardiac and vascular malformations [117]. Moreover, HIF-1α is associated with coordinating energy availability and utilization in the heart and has a central role in balancing oxygen demand and supply. Cardiomyocyte-specific HIF-1α gene deletion in the hearts of genetically engineered mice caused a significant reduction in contractility and vascularization, and is accompanied with altered expression of genes involved in angiogenesis and glucose metabolism [118]. After permanent coronary artery ligation in rats, HIF-1α and HIF-2α accumulate at the borderzone of the infarcted tissue, in nuclei of cardiomyocytes, interstitial cells and endothelial cells. This persists for four weeks and is colocalized with transcriptional target gene expression [119].
Therapy based on expression of HIF can be regarded as a strategy to induce neo-angiogenesis in the ischemic heart. Administration of HIF-1α, by intramyocardial injection of HIF-1α encoding plasmid DNA in a permanent ligation infarction model in the rat, significantly decreased infarct size and enhanced neovascularisation by increasing capillary density and thereby regional myocardial blood flow. [120] Therapeutic interventions aimed at the increase the endogenous HIF-1 expression, can be accomplished by blockade of degradation of HIF-1α [121] or by the use of small molecule inhibitors of the HIF-hydroxylases [122,123]. Interestingly, HIF activates gene expression of several additional vasculogenic growth factors besides VEGF. Transgenic mice containing constitutively active HIF-1α molecule showed significantly increased activation of HIF transcriptional targets and hypervascularity. These vessels were not associated with increased edema and their vascular integrity appeared to be fully intact, in contrast to phenotypes developing in transgenic mice overexpressing only VEGF [124]. Therefore, targeting HIF instead of VEGF can activate more angiogenic factors at the same time resulting in intact neo-vascularization.
To summarize, HIF is a hypoxia-sensitive transcription factor, which is able to orchestrate and activate many factors and pathways that are indispensable after ischemic damage. Early overexpression of HIF can result in an increased transcriptional response of factors involved in pathways that increase oxygen delivery and promote adaptive pro-survival responses, which is substantial for stem cell mediated cardiac repair.

H. Vascular Endothelial Growth Factor

Vascular Endothelial Growth Factor (VEGF) is a group of secreted proteins that are produced by almost every cell type [125]. Embryos lacking just one allele of VEGF-A (VEGF $^{+/-}$ mice) are lethal because of abnormal blood vessel development [126]. Also both VEGF-A receptors, VEGF-R1 (flt-1) and VEGF-R2 (Flk-1 or KDR), are important for endothelial differentiation, migration, proliferation and vascular remodeling, as can be concluded from knockout studies [127]. Cultured rat cardiomyocytes subjected to hypoxia rapidly induced mRNA expression of VEGF, which is also observed in vivo in the ischemic myocardium [128].
VEGF is a strong promoter of angiogenesis, which is illustrated by clinical studies with myocardial infarction patients. Naked plasmid DNA encoding VEGF-165 directly injected into the ischemic myocardium in patients with symptomatic myocardial ischemia, led to

reduced symptoms and improved myocardial perfusion [129-131]. Beside the capability of VEGF to promote neo-vascularization, which is investigated thoroughly (see for review [132]), VEGF is also involved in the mobilization of BMSCs [133,134]. In a clinical study of patients with acute myocardial infarction, increased plasma levels of VEGF correlated significantly with an increase in circulating BMSCs (CD34+), which indicates that VEGF is able to recruit stem cells in myocardial infarction patients [135].
The importance of VEGF in stem cell-mediated therapy after myocardial infarction, is further illustrated by a recent study in which intravenous injection of BMSC after coronary artery ligation in mice was performed. The authors showed that the decrease in infarct size, caused by the BMSC injection, was diminished after blocking VEGF with either neutralizing antibodies or with gene transfer of a soluble form of the VEGF-R1 receptor [136]. Moreover, in another study, even 2 month after intramyocardial injection of BMSCs into a one week old myocardial infarction, elevated levels of VEGF were observed together with improved perfusion and cardiac function. The assumed underlying mechanism is para-secretion of growth factors paralleled by the differentiation of BMSCs into endothelial cells [137]. Beside the BMSC mobilizing capacity of VEGF, this factor may also be involved in induction of cardiomyocyte proliferation, although there is not much conclusive evidence. Intramyocardial injection of a naked plasmid DNA encoding VEGF-165 in pigs, who underwent coronary occlusion, resulted in a several-fold increase in number of mitotic cardiomyocyte nuclei and nuclear hyperplasia, suggesting that VEGF could either directly or indirectly promote karyokinesis in cardiomyocytes [138].
To summarize, VEGF is involved in stem cell mediated cardiac repair, because of its prominent role in angiogenesis, but also its capability of mobilizing BMSC into the peripheral blood in myocardial infarction patients. Furthermore, it might act as a mitogen on cardiomyocytes.

I. EPO

he production of Erythropoietin (EPO) is induced by hypoxia and is predominantly produced by the kidneys in adult life [139]. Other cell types, such as activated macrophages, also express EPO mRNA [140], which could play a role in the inflammatory reaction after myocardial infarction since this is accompanied by a massive invasion of macrophages. EPO-R, the Erythropoietin Receptor, is expressed in the heart mainly in the epicardium and pericardium on endothelial cells, smooth muscle cells and cardiomyocytes [141].
EPO is not only associated with erythropoiesis, it also plays a crucial role in cardiac development. EPO and EPO receptor knockout mice ($EPO^{-/-}$ and $EPO\text{-}R^{-/-}$ mice) are both embryonic lethal, due to a combination of anemia and cardiac abnormalities. In these mice a ventricular hypoplasia exists, potentially due to a reduction in the number of proliferating cardiomyocytes [141]. Probably, this developmental cardiac abnormality is due to altered hematopoietic expression of EPO-R, since Suzuki et al. showed that in transgenic mice that expressed EPO-R exclusively in the hematopoietic lineage (so they lack EPO-R expression in non-haematopoietic tissue), normal cardiac development occured [142].
In vitro, neonatal rat cardiomyocytes mitotically respond in a dose-dependant fashion to recombinant Human EPO (rHuEPO), which could be blocked with antibodies against Human EPO. Thus, EPO appears to be a strong mitogen for neonatal cardiomyocytes [143]. Moreover, cultured adult rat cardiomyocytes subjected to hypoxia were prevented from apoptosis by EPO. In vivo, 7 subsequent daily EPO injections started directly after coronary ischemia-reperfusion in the rat reduced cardiomyocyte loss by 50% [144]. In addition, recombinant Human EPO stimulated capillary outgrowth in myocardial tissue in

an in vitro angiogenesis assay using adult human myocardial tissue [145].
Notwithstanding positive cardiac effects of EPO, we have to bear in mind that an excess of EPO leads to an elevation in blood viscosity and thrombolic events. Transgenic mice overexpressing human EPO have an increased ventricular dilatation and have intracellular edema of the cardiomyocytes, which results in cardiac dysfunction, reduced exercise performance and a significant shorter life expectancy [146,147]. Thus, although EPO has various positive effects on cardiac repair, an overload of EPO has quite the opposite effects.
Finally, EPO is also involved in proliferation and mobilization of BMSCs. EPO treated mice showed an increase in number and in proliferation of BMSC in the bone marrow and an increase in circulating Endothelial Progenitor cells, which contributed to significantly improved ischemia-induced neovascularization [148]. This is in concordance with the raised serum levels of EPO in patients with coronary artery disease that was associated with the number and function of circulating EPC [148].
In conclusion, EPO is a potent factor in stem cell-mediated repair. EPO has anti-apoptotic properties and is involved in cardiomyocyte proliferation. EPO stimulates neovascularization and has proliferative and mobilizing effects on BMSCs.

7. Conclusion

A plethora of cytokines and growth factors appear to play a role in stem cell-mediated cardiac repair of the infarcted myocardium. In this process, it is difficult to pinpoint the function and efficacy of each individual factor, due to pleiotrophic, redundant, synergistic and antagonistic properties of orchestrating factors and their, often undefined, concomitant receptors. Most importantly, the effects are time and spatial dependent. We are only beginning to elucidate the role and significance of cytokines and growth factors in the communication for stem cell-mediated cardiac repair (see table 1).

Table 1
Summary of effects of a selection of signaling factors on the different steps in stem cell-mediated cardiac repair

Factor	Post-MI	Inflammation	Mobilization	Homing	Survival	Proliferation	Differentiation into CM	Angiogenesis	Hazardous effects
TNF-α	↑	↑	+	N.D.	N.D.	–	N.D.	+	Inducing heart failure
IL-8	↑	↑	+	N.D.	N.D.	N.D.	N.D.	+	Neutrophil attractant
G-CSF	–	↑	++	N.D.	+	+	+?	+	Reduction of HSC activity
SCF	↓	↑	++	+	+	+	+?	+	N.D.
SDF-1	↑	N.D.	+	++	+	N.D.	+?	+	N.D.
HGF	↑	N.D.	–	+	+	+	+?	+	N.D.
HIF-1α	↑	↑	N.D.	N.D.	+	N.D.	N.D.	+	N.D.
VEGF	↑	↑	+	+	+	N.D.	+?	+	Leaky vessels
EPO	↑	↑	+	N.D.	+	+	+?	+	Excess provokes MI

N.D. = not described; ↑: increased; ↓: decreased; +: improved; – worsened.

To gain the optimal regenerative capacity for clinical application, we need to target and balance the orchestrating factors that play a crucial role in the fundamental steps of stem cell mediated-cardiac repair: mobilization, incorporation, survival, differentiation and proliferation. Affecting the orchestrating factors could be either by enhancing or blocking their presence, depending on their spatial-dependant separate roles.
Whether mobilizing stem cells from the bone marrow can be circumvented by directly aiming at the small but evident cardiac stem cell population, is under investigation.
To induce stem cell-medicated cardiac repair, the 3 recurrent substantial components

should be targeted. 1) The bone marrow as major stem cell reservoir should be triggered to release BMSCs, preferably cardiac progenitor cells. 2) The peripheral circulation as transport way and infrastructure within the infarcted myocardium should be optimal to home and to incorporate BMSCs in the infarcted zone. 3) The infarcted myocardium itself, the place of repair, should be attractive and inviting for BMSCs.
There should be space to incorporate, BMSC should not be challenged by the infiltrated inflammatory cells and inflammatory cytokines to differentiate into leukocytes, and there should be factors present that give liberty to BMSCs to proliferate and differentiate into cardiomyocytes and vascular cells (Fig. 2). The regulation between these components should be done by mobilizing factors, such a G-CSF, SCF and SDF-1. Regulation within the infarct in the acute phase is primarily by (pro-) inflammatory cytokines (among which TNF-α and IL-8) of which negative effects should be avoided. Positive effects on angiogenesis and myogenesis could be expected from HIF-1α, HGF, VEGF and EPO.
Altogether, the possibilities of stem cell-mediated cardiac repair seem promising and are starting to put an end to the era of the insurmountable consequences of myocardial infarction.

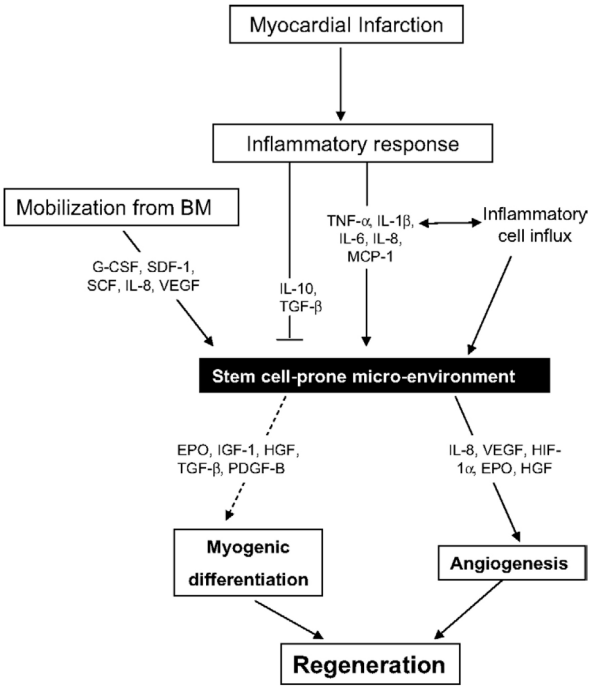


Figure 2. *Overview of the key processes and factors involved in the stem cell-mediated cardiac repair process. Myocardial infarction is followed by an inflammatory response in which the interplay of inflammatory factors and influxes of various cell types play a decisive role for the subsequent regeneration. Ideally a beneficial stem cell prone environment will develop in which BMSCs or CSCs are attracted and activitated such that and regeneration is augmented while scarring is attenuated. This requires a proper balance of secreted mediators.*

References

[1] Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? Cell 2001; 105: 829-841.

[2] Fuchs E, Segre JA. Stem cells: a new lease on life. Cell 2000; 100: 143-155.

[3] Kuehnle I, Goodell MA. The therapeutic potential of stem cells from adults. BMJ 2002; 325: 372-376.

[4] Dowell JD, Rubart M, Pasumarthi KB, Soonpaa MH, Field LJ. Myocyte and myogenic stem cell transplantation in the heart. Cardiovasc Res 2003; 58: 336-350.

[5] Orlic D, Hill JM, Arai AE. Stem cells for myocardial regeneration. Circ Res 2002; 91: 1092-1102.

[6] Mathur A, Martin JF. Stem cells and repair of the heart. Lancet 2004; 364: 183-192.

[7] Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 2002; 297: 2256-2259.

[8] Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003; 425: 968-973.

[9] Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci U S A 2003; 100: 12313-12318.

[10] Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004; 428: 664-668.

[11] Yoder MC, Williams DA. Matrix molecule interactions with hematopoietic stem cells. Experimental hematology 1995; 23: 961.

[12] Papayannopoulou T, Nakamoto B. Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. Proc Natl Acad Sci U S A 1993; 90: 9374-9378.

[13] Vermeulen M, Le Pesteur F, Gagnerault MC, Mary JY, Sainteny F, Lepault F. Role of adhesion molecules in the homing and mobilization of murine hematopoietic stem and progenitor cells. Blood 1998; 92: 894-900.

[14] Frenette PS, Mayadas TN, Rayburn H, Hynes RO, Wagner DD. Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. Cell 1996; 84: 563-574.

[15] Kucia M, Dawn B, Hunt G, Guo Y, Wysoczynski M, Majka M, Ratajczak J, Rezzoug F, Ildstad ST, Bolli R, Ratajczak MZ. Cells expressing early cardiac markers reside in the bone marrow and are mobilized into the peripheral blood after myocardial infarction. Circ Res 2004; 95: 1191-1199.

[16] Ratajczak MZ, Kucia M, Reca R, Majka M, Janowska-Wieczorek A, Ratajczak J. Stem cell plasticity revisited: CXCR4-positive cells expressing mRNA for early muscle, liver and neural cells 'hide out' in the bone marrow. Leukemia 2004; 18: 29-40.

[17] Lapidot T, Petit I. Current understanding of stem cell mobilization: the roles of chemokines,proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Experimental hematology 2002; 30: 973.

[18] Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P. Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 2001; 344: 1750-1757.

[19] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, ChimentiS, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114: 763-776.

[20] Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. N Engl J Med 2002; 346: 5-15.

[21] Hocht-Zeisberg E, Kahnert H, Guan K, Wulf G, Hemmerlein B, Schlott T, Tenderich G, Korfer R, Raute-Kreinsen U, Hasenfuss G. Cellular repopulation of myocardial infarction in patients with sex-mismatched heart transplantation. Eur Heart J 2004; 25: 749-758.
[22] Deb A, Wang S, Skelding KA, Miller D, Simper D, Caplice NM. Bone marrow-derived cardiomyocytes are present in adult human heart: A study of gender-mismatched bone marrow transplantation patients. Circulation 2003; 107: 1247-1249.
[23] Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001; 107: 1395-1402.
[24] Kuramochi Y, Fukazawa R, Migita M, Hayakawa J, Hayashida M, Uchikoba Y, Fukumi D, Shimada T, Ogawa S. Cardiomyocyte regeneration from circulating bone marrow cells in mice. Pediatr Res 2003; 54: 319-325.
[25] Blankesteijn WM, Creemers E, Lutgens E, Cleutjens JP, Daemen MJ, Smits JF. Dynamics of cardiac wound healing following myocardial infarction: observations in genetically altered mice. Acta Physiol Scand 2001; 173: 75-82.
[26] Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C, Tincey S, Michael LH, Entman ML, Frangogiannis NG. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. Am J Pathol 2004; 164: 665-677.
[27] Birdsall HH, Green DM, Trial J, Youker KA, Burns AR, MacKay CR, LaRosa GJ, Hawkins HK, Smith CW, Michael LH, Entman ML, Rossen RD. Complement C5a, TGF-beta, and MCP-1, in sequence, induce migration of monocytes into ischemic canine myocardium within the first one to five hours after reperfusion. Circulation 1997; 95: 684-692.
[28] Dreyer WJ, Michael LH, Nguyen T, Smith CW, Anderson DC, Entman ML, Rossen RD. Kinetics of C5a release in cardiac lymph of dogs experiencing coronary artery ischemia-reperfusion injury. Circ Res 1992; 71: 1518-1524.
[29] Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. Cardiovasc Res 2000; 47: 446-456.
[30] Kaminski KA, Bonda TA, Korecki J, Musial WJ. Oxidative stress and neutrophil activation--the two keystones of ischemia/reperfusion injury. Int J Cardiol 2002; 86: 41-59.
[31] Lefer DJ, Granger DN. Oxidative stress and cardiac disease. Am J Med 2000; 109: 315-323.
[32] Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002; 53: 31- 47.
[33] Frangogiannis NG, Lindsey ML, Michael LH, Youker KA, Bressler RB, Mendoza LH, Spengler RN, Smith CW, Entman ML. Resident cardiac mast cells degranulate and release preformed TNF-alpha, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation 1998; 98: 699-710.
[34] Bozkurt B, Kribbs SB, Clubb FJ, Jr., Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodelingin rats. Circulation 1998; 97: 1382-1391.
[35] Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, Demetris AJ, Feldman AM. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-alpha. Circ Res 1997; 81: 627-635.
[36] Thaik CM, Calderone A, Takahashi N, Colucci WS. Interleukin-1beta modulates the growth and phenotype of neonatal rat cardiac myocytes. J Clin Invest 1995; 96: 1093-1099.
[37] Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. Circ Res 2002; 91: 988-998.
[38] Ivey CL, Williams FM, Collins PD, Jose PJ, Williams TJ. Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit. Evidence for a role for C5a and interleukin-8. J Clin Invest 1995; 95: 2720-2728.
[39] Sekido N, Mukaida N, Harada A, Nakanishi I, Watanabe Y, Matsushima K. Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. Nature

1993; 365: 654-657.
[40] Mosmann TR. Properties and functions of interleukin-10. Adv Immunol 1994; 56: 1-26.
[41] Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992; 267: 10931-10934.
[42] Shi Q, Rafii S, Wu MH, Wijelath ES, Yu C, Ishida A, Fujita Y, Kothari S, Mohle R, Sauvage LR, Moore MA, Storb RF, Hammond WP. Evidence for circulating bone marrow-derived endothelial cells. Blood 1998; 92: 362-367.
[43] Asahara T, Murohara T, Sullivan A, Silver M, van der ZR, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative pro-genitor endothelial cells for angiogenesis. Science 1997; 275: 964-967.
[44] Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999; 5: 434-438.
[45] Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, Schaper W. Bone marrow-derived cells do not incorporate into the adult growing vasculature. Circ Res 2004; 94: 230-238.
[46] Weihrauch D, Arras M, Zimmermann R, Schaper J. Importance of monocytes/ macrophages and fibroblasts for healing of micronecroses in porcine myocardium. Molecular and cellular biochemistry 1995; 147: 13.
[47] Jacobs M, Staufenberger S, Gergs U, Meuter K, Brandstatter K, Hafner M, Ertl G, Schorb W. Tumor necrosis factor-alpha at acute myocardial infarction in rats and effects on cardiac fibroblasts. Journal of molecular and cellular cardiology 1999; 31: 1949.
[48] Kapadia S, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. J Clin Invest 1995; 96: 1042-1052.
[49] Bryant D, Becker L, Richardson J, Shelton J, Franco F, Peshock R, Thompson M, Giroir B. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha. Circulation 1998; 97: 1375-1381.
[50] Sivasubramanian N, Coker ML, Kurrelmeyer KM, MacLellan WR, DeMayo FJ, Spinale FG, Mann DL. Left ventricular remodeling in transgenic mice with cardiac restricted overexpression of tumor necrosis factor. Circulation 2001; 104: 826-831.
[51] Berthonneche C, Sulpice T, Boucher F, Gouraud L, de Leiris J, O'Connor SE, Herbert JM, Janiak P. New insights into the pathological role of TNF-alpha in early cardiac dysfunction and subsequent heart failure after infarction in rats. Am J Physiol Heart Circ Physiol 2004; 287: H340-H350.
[52] Dawn B, Guo Y, Rezazadeh A, Wang OL, Stein AB, Hunt G, Varma J, Xuan YT, Wu WJ, Tan W, Zhu X, Bolli R. Tumor necrosis factor-alpha does not modulate ischemia/reperfusion injury in naive myocardium but is essential for the development of late precondi tioning. J Mol Cell Cardiol 2004; 37: 51-61.
[53] Maekawa N, Wada H, Kanda T, Niwa T, Yamada Y, Saito K, Fujiwara H, Sekikawa K, Seishima M. Improved myocardial ischemia/reperfusion injury in mice lacking tumor necrosis factor-alpha. J Am Coll Cardiol 2002; 39: 1229-1235.
[54] Sugano M, Tsuchida K, Hata T, Makino N. In vivo transfer of soluble TNF-alpha receptor 1 gene improves cardiac improves cardiac function and reduces infarct size after myocardial infarction in rats. The FASEB journal 2004; 18: 911.
[55] Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, Djian J, Drexler H, Feldman A, Kober L, Krum H, Liu P, Nieminen M, Tavazzi L, van Veldhuisen DJ, Waldenstrom A, Warren M, Westheim A, Fleming T. Targeted anticytokine therapy in patients with chronic heart failure:results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation 2004; 109: 1594.
[56] Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT, Anti-TNF Therapy Against Congestive Heart Failure Investigators. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in

patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003; 107: 3133.
[57] Kurrelmeyer KM, Michael LH, Baumgarten G, Taffet GE, Peschon JJ, Sivasubramanian N, Entman ML, Mann DL. Endogenous tumor necrosis factor protects the adult cardiac myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. Proc Natl Acad Sci U S A 2000; 97: 5456-5461.
[58] Nakano M, Knowlton AA, Dibbs Z, Mann DL. Tumor necrosis factor-alpha confers resistance to hypoxic injury in the adult mammalian cardiac myocyte. Circulation 1998; 97: 1392-1400.
[59] Chen Y, Ke Q, Yang Y, Rana JS, Tang J, Morgan JP, Xiao YF. Cardiomyocytes overexpressing TNF-alpha attract migration of embryonic stem cells via activation of p38 and c-Jun amino-terminal kinase. FASEB J 2003; 17: 2231-2239.
[60] Zhang Y, Harada A, Bluethmann H, Wang JB, Nakao S, Mukaida N, Matsushima K. Tumor necrosis factor (TNF) is a physiologic regulator of hematopoietic progenitor cells: increase of early hematopoietic progenitor cells in TNF receptor p55-deficient mice in vivo and potent inhibition of progenitor cell proliferation by TNF alpha in vitro. Blood 1995; 86: 2930-2937.
[61] Valgimigli M, Rigolin GM, Fucili A, Porta MD, Soukhomovskaia O, Malagutti P, Bugli AM, Bragotti LZ, Francolini G, Mauro E, Castoldi G, Ferrari R. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. Circulation 2004; 110: 1209-1212.
[62] Jeannin P, Delneste Y, Gosset P, Molet S, Lassalle P, Hamid Q, Tsicopoulos A, Tonnel AB. Histamine induces interleukin-8 secretion by endothelial cells. Blood 1994; 84: 2229-2233.
[63] Kukielka GL, Smith CW, LaRosa GJ, Manning AM, Mendoza LH, Daly TJ, Hughes BJ, Youker KA, Hawkins HK, Michael LH, . Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. J Clin Invest 1995; 95: 89-103.
[64] Laterveer L, Lindley IJ, Hamilton MS, Willemze R, Fibbe WE. Interleukin-8 induces rapid mobilization of hematopoietic stem cells with radioprotective capacity and long-term myelolymphoid repopulating ability. Blood 1995; 85: 2269-2275.
[65] Fibbe WE, Pruijt JF, van Kooyk Y, Figdor CG, Opdenakker G, Willemze R. The role of metalloproteinases and adhesion molecules in interleukin-8-induced stem-cell mobilization. Semin Hematol 2000; 37: 19-24.
[66] Watanabe T, Kawano Y, Kanamaru S, Onishi T, Kaneko S, Wakata Y, Nakagawa R, Makimoto A, Kuroda Y, Takaue Y, Talmadge JE. Endogenous interleukin-8 (IL-8) surge in granulocyte colony-stimulating factor-induced peripheral blood stem cell mobilization. Blood 1999; 93: 1157-1163.
[67] Laterveer L, Lindley IJ, Heemskerk DP, Camps JA, Pauwels EK, Willemze R, Fibbe WE. Rapid mobilization of hematopoietic progenitor cells in rhesus monkeys by a single intravenous injection of interleukin-8. Blood 1996; 87: 781-788.
[68] Pruijt JF, Fibbe WE, Laterveer L, Pieters RA, Lindley IJ, Paemen L, Masure S, Willemze R, Opdenakker G. Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9). Proc Natl Acad Sci U S A 1999; 96: 10863-10868.
[69] Pruijt JF, Verzaal P, van Os R, de Kruijf EJ, van Schie ML, Mantovani A, Vecchi A, Lindley IJ, Willemze R, Starckx S, Opdenakker G, Fibbe WE. Neutrophils are indispensable for hematopoietic stem cell mobilization induced by interleukin-8 in mice. Proc Natl Acad Sci U S A 2002; 99: 6228-6233.
[70] Kohtani T, Abe Y, Sato M, Miyauchi K, Kawachi K. Protective effects of anti-neutrophil antibody against myocardial ischemia/reperfusion injury in rats. Eur Surg Res 2002; 34: 313-320.
[71] Metcalf D, Nicola NA. Proliferative effects of purified granulocyte colony-stimulating factor (G-CSF) on normal mouse hemopoietic cells. J Cell Physiol 1983; 116: 198-206.
[72] Anderson DM, Lyman SD, Baird A, Wignall JM, Eisenman J, Rauch C, March CJ,

Boswell HS, Gimpel SD, Cosman D, . Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell 1990; 63: 235-243.

[73] Ulich TR, del Castillo J, Yi ES, Yin S, McNiece I, Yung YP, Zsebo KM. Hematologic effects of stem cell factor in vivo and in vitro in rodents. Blood 1991; 78: 645-650.

[74] Kim CH, Broxmeyer HE. In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: stromalcell-derived factor-1, steel factor, and the bone marrow environment. Blood 1998; 91: 100-110.

[75] Gersch C, Dewald O, Zoerlein M, Michael LH, Entman ML, Frangogiannis NG. Mast cells and macrophages in normal C57/BL/6 mice. Histochem Cell Biol 2002; 118: 41-49.

[76] Woldbaek PR,Hoen IB, Christensen G, Tonessen T. Gene expression of colony-stimulating factors and stem cell factor after myocardial infarction in the mouse. Acta Physiologica Scandinavica 2002; 175: 173-181.

[77] Verfaillie CM. Hematopoietic stem cells for transplantation. Nature immunology 2002; 3: 314.

[78] Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410: 701-705.

[79] Norol F, Merlet P, Isnard R, Sebillon P, Bonnet N, Cailliot C, Car-rion C, Ribeiro M, Charlotte F, Pradeau P, Mayol JF, Peinnequin A, Drouet M, Vernant JP, Herodin F. Influence of mobilized stem cells on myocardial infarct repair in a non human primate model. Blood 2003; 102: 4361.

[80] Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003; 362: 697-703.

[81] Ohtsuka M, Takano H, Zou Y, Toko H, Akazawa H, Qin Y, Suzuki M, Hasegawa H, Nakaya H, Komuro I. Cytokine therapy prevents left ventricular remodeling and dysfunction after myocardial infarction through neovascularization. The FASEB journal 2004; 18: 851.

[82] Frangogiannis NG, Perrard JL, Mendoza LH, Burns AR, Lindsey ML, Ballantyne CM, Michael LH, Smith CW, Entman ML. Stem-cell factor induction is associated with mast cell accumulation after canine myocardial ischemia and reperfusion. Circulation 1998; 98: 687-698.

[83] Suda T, Suda J, Kajigaya S, Nagata S, Asano S, Saito M, Miura Y. Effects of recombinant murine granulocyte colony-stimulating factor on granulocyte-macrophage and blast colony formation. Exp Hematol 1987; 15: 958-965.

[84] Minatoguchi S, Takemura G, Chen XH, Wang N, Uno Y, Koda M, Arai M, Misao Y, Lu C, Suzuki K, Goto K, Komada A, Takahashi T, Kosai K, Fujiwara T, Fujiwara H. Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by postinfarction granulocyte colony-stimulating factor treatment. Circulation 2004; 109: 2572-2580.

[85] Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, Kim YJ, Soo LD, Sohn DW, Han KS, Oh BH, Lee MM, Park YB. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 2004; 363: 751-756.

[86] Petzsch M, Ince H, Kleine HD, Schmidt H, Rehders T, Körber T, Freund M, Nienaber C. 2004. No Restenosis after G-CSF in Acute Myocardial Infarction: Insights from FIRSTLINE-AMI (Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor) (abstract). Annual Scientific Sessions of the American Heart Association 2004: 1140.

[87] Ince H, Petsch M, Kleine HD, Smidt H, Rehders T, Korber T, Chatterjee T, Freund M, Nienaber C. 2004. Prevention of LV Remodeling with G-CSF in Acute Myocardial

Infarction: Insights from FIRSTLINE-AMI (Front-Integrated Revascularization and Stem- Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor) (abstract). Annual Scientific Sessions of the American Heart Association 2004: 1680/C72.

[88] Franz WM, Theiss HD, Hennig C, Wintersperger BJ, Huber A, Schoenberg SO, Engelmann M.G., Maximilian L. 2004. Bone-Marrow Stem-Cell Mobilization Induced by Granulocyte-Colony Stimulating Factor (G-CSF) in Patients Undergoing Delayed Revascularization for ST-Segment Elevation Myocardial Infarction (abstract).Annual Scientific Sessions of the American Heart Association2004: 1554.

[89] Ripa R.S., Yongzhong W, Jørgensen E, Kastrup J. 2004. Bone-Marrow Stem Cell Mobilization Induced by Granulocyte-Colony Stimulating Factor in Patients with a PCI-treated Acute ST-Elevation Myocardial Infarction is Safe: Results from a Double-blind Randomized Study, the Stem Cells in Myocardial Infarction (STEMMI) Trial (abstract). Annual Scientific Sessions of the American Heart Association 2004: 1139.

[90] Honold J, Lehmann R, Assmus B, Martin H, Walter DH. 2005. Granulocyte Colony Stimulating Factor(G-CSF) -Induced Mobilization Profoundly Impairs Functional Activities of Circulating Endothelial Progenitor Cells in Patients with Chronic Ischemic Heart Disease (abstract). Annual Scientific Sessions of the AmericaN Heart Association 2004: 240.

[91] Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, Ogawa S, Okano H, Hotta T, Ando K, Fukuda K. Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 2004; 104: 3581-3587.

[92] Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, Bosch-Marce M, Masuda H, Losordo DW, Isner JM, Asahara T. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation 2003; 107: 1322-1328.

[93] Hattori K, Heissig B, Tashiro K, Honjo T, Tateno M, Shieh JH, Hackett NR, Quitoriano MS, Crystal RG, Rafii S, Moore MA. Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. Blood 2001; 97: 3354-3360.

[94] Moore MA, Hattori K, Heissig B, Shieh JH, Dias S, Crystal RG, Rafii S. Mobilization of endothelial and hematopoietic stem and progenitor cells by adenovector-mediated elevation of serum levels of SDF-1, VEGF, and angiopoietin-1. Ann N Y Acad Sci 2001; 938: 36-45.

[95] Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, Ponomaryov T, Taichman RS, Arenzana-Seisdedos F, Fujii N, Sandbank J, Zipori D, Lapidot T. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol 2002; 3: 687-694.

[96] Kahn J, Byk T, Jansson-Sjostrand L, Petit I, Shivtiel S, Nagler A, Hardan I, Deutsch V, Gazit Z, Gazit D, Karlsson S, Lapidot T. Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. Blood 2004; 103: 2942-2949.

[97] Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 2002; 109: 625-637.

[98] Levesque JP, Liu F, Simmons PJ, Betsuyaku T, Senior RM, Pham C, Link DC. Characterization of hematopoietic progenitor mobilization in protease-deficient mice. Blood 2004; 104: 65-72.

[99] Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996; 382: 635-638.

[100] Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine

receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998; 393: 595-599.
[101] Pillarisetti K, Gupta SK. Cloning and relative expression analysis of rat stromal cell derived factor-1 (SDF-1)1: SDF-1 alpha mRNA is selectively induced in rat model of myocardial infarction. Inflammation 2001; 25: 293-300.
[102] Heeschen C, Lehmann R, Honold J, Assmus B, Aicher A, Walter DH, Martin H, Zeiher AM, Dimmeler S. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation 2004; 109: 1615-1622.
[103] Zarnegar R, Michalopoulos GK. The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis. J Cell Biol 1995; 129: 1177-1180.
[104] Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. J Biochem (Tokyo) 1996; 119: 591-600.
[105] Fujii K, Ishimaru F, Kozuka T, Matsuo K, Nakase K, Kataoka I, Tabayashi T, Shinagawa K, Ikeda K, Harada M, Tanimoto M. Elevation of serum hepatocyte growth factor during granulocyte colony-stimulating factor-induced peripheral blood stem cell mobilization. Br J Haematol 2004; 124: 190-194.
[106] Rappolee DA, Iyer A, Patel Y. Hepatocyte growth factor and its receptor are expressed in cardiac myocytes during early cardiogenesis. Circ Res 1996; 78: 1028-1036.
[107] Ueda H, Nakamura T, Matsumoto K, Sawa Y, Matsuda H, Nakamura T. A potential cardioprotective role of hepatocyte growth factor in myocardial infarction in rats. Cardiovasc Res 2001; 51: 41-50.
[108] Nakamura T, Mizuno S, Matsumoto K, Sawa Y, Matsuda H, Nakamura T. Myocardial protection from ischemia/reperfusion injury by endogenous and exogenous HGF. J Clin Invest 2000; 106: 1511-1519.
[109] Funatsu T, Sawa Y, Ohtake S, Takahashi T, Matsumiya G, Matsuura N, Nakamura T, Matsuda H. Therapeutic angiogenesis in the ischemic canine heart induced by myocardial injection of naked complementary DNA plasmid encoding hepatocyte growth factor. J Thorac Cardiovasc Surg 2002; 124: 1099-1105.
[110] Jayasankar V, Woo YJ, Bish LT, Pirolli TJ, Chatterjee S, Berry MF, Burdick J, Gardner TJ, Sweeney HL. Gene transfer of hepatocyte growth factor attenuates postinfarction heart failure. Circulation 2003; 108 Suppl 1: II230-II236.
[111] Miyagawa S, Sawa Y, Taketani S, Kawaguchi N, Nakamura T, Matsuura N, Matsuda H. Myocardial regeneration therapy for heart failure: hepatocyte growth factor enhances the effect of cellular cardiomyoplasty. Circulation 2002; 105: 2556-2561.
[112] Weimar IS, Miranda N, Muller EJ, Hekman A, Kerst JM, de Gast GC, Gerritsen WR. Hepatocyte growth factor/scatter factor (HGF/SF) is produced by human bone marrow stromal cells and promotes proliferation, adhesion and survival of human hematopoietic progenitor cells (CD34+). Exp Hematol 1998; 26: 885-894.
[113] Duan HF, Wu CT, Wu DL, Lu Y, Liu HJ, Ha XQ, Zhang QW, Wang H, Jia XX, Wang LS. Treatment of myocardial ischemia with bone marrow-derived mesenchymal stem cells overexpressing hepatocyte growth factor. Mol Ther 2003; 8: 467-474.
[114] Semenza GL. Hypoxia-inducible factor 1: control of oxygen homeostasis in health and disease. Pediatric research 2001; 49: 614.
[115] Kakinuma Y, Miyauchi T, Yuki K, Murakoshi N, Goto K, Yamaguchi I. Novel molecular mechanism of increased myocardial endothelin-1 expression in the failing heart involving the transcriptional factor hypoxia-inducible factor-1alpha induced for impaired myocardial energy metabolism. Circulation 2001; 103: 2387.
[116] Cai Z, Manalo DJ, Wei G, Rodriguez ER, Fox-Talbot K, Lu H, Zweier JL, Semenza GL. Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. Circulation 2003; 108: 79.
[117] Compernolle V, Brusselmans K, Franco D, Moorman A, Dewerchin M, Collen D, Carmeliet P. Cardia bifida, defective heart development and abnormal neural crest migration

in embryos lacking hypoxia-inducible factor-1alpha. Cardiovascular research 2003; 60: 569.
[118] Huang Y, Hickey RP, Yeh JL, Liu D, Dadak A, Young LH, Johnson RS, Giordano FJ. Cardiac myocyte-specific HIF-1alpha deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart. The FASEB journal 2004; 18: 1138.
[119] Jurgensen JS, Rosenberger C, Wiesener MS, Warnecke C, Horstrup JH, Grafe M, Philipp S, Griethe W, Maxwell PH, Frei U, Bachmann S, Willenbrock R, Eckardt KU. Persistent induction of HIF-1alpha and -2alpha in cardiomyocytes and stromal cells of ischemic myocardium. FASEB J 2004.
[120] Shyu K, Wang MT, Wang BW, Chang CC, Leu JG, Kuan P, Chang H. Intramyocardial injection of naked DNA encoding HIF-1alpha/VP16 hybrid to enhance angiogenesis in an acute myocardial infarction model in the rat. Cardiovascular research 2002; 54: 576.
[121] Willam C, Masson N, Tian YM, Mahmood SA, Wilson MI, Bicknell R, Eckardt KU, Maxwell PH, Ratcliffe PJ, Pugh CW. Peptide blockade of HIFalpha degradation modulates cellular metabolism and angiogenesis. Proc Natl Acad Sci U S A 2002; 99: 10423-10428.
[122] Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001; 107: 43-54.
123] Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG, Jr. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 2001; 292: 464-468.
[124] Elson DA, Thurston G, Huang LE, Ginzinger DG, McDonald DM, Johnson RS, Arbeit JM. Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1alpha. Genes Dev 2001; 15: 2520-2532.
[125] Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989; 246: 1306-1309.
[126] Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996; 380: 435-439.
[127] Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995; 376: 66-70.
[128] Banai S, Shweiki D, Pinson A, Chandra M, Lazarovici G, Keshet E. Upregulation of vascular endothelial growth factor expression induced by myocardial ischaemia: implications for coronary angiogenesis. Cardiovasc Res 1994; 28: 1176-1179.
[129] Losordo DW, Vale PR, Symes JF, Dunnington CH, Esakof DD, Maysky M, Ashare AB, Lathi K, Isner JM. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. Circulation 1998; 98: 2800-2804.
[130] Sarkar N, Ruck A, Kallner G, Hassan S, Blomberg P, Islam KB, van der LJ, Lindblom D, Nygren AT, Lind B, Brodin LA, Drvota V, Sylven C. Effects of intramyocardial injection of phVEGF-A165 as sole therapy in patients with refractory coronary artery disease--12-month follow-up: angiogenic gene therapy. J Intern Med 2001; 250: 373-381.
[131] Symes JF, Losordo DW, Vale PR, Lathi KG, Esakof DD, Mayskiy M, Isner JM. Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease. Ann Thorac Surg 1999; 68: 830-836.
[132] Simons M, Ware JA. Therapeutic angiogenesis in cardiovascular disease. Nat Rev Drug Discov 2003; 2: 863-871.
[133] Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived

endothelial progenitor cells. EMBO J 1999; 18: 3964-3972.
[134] Hattori K, Dias S, Heissig B, Hackett NR, Lyden D, Tateno M, Hicklin DJ, Zhu Z, Witte L, Crystal RG, Moore MA, Rafii S. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J Exp Med 2001; 193: 1005-1014.
[135] Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. Circulation 2001; 104: 1046-1052.
[136] Hiasa K, Egashira K, Kitamoto S, Ishibashi M, Inoue S, Ni W, Zhao Q, Nagata S, Katoh M, Sata M, Takeshita A. Bone marrow mononuclear cell therapy limits myocardial infarct size through vascular endothelial growth factor. Basic Res Cardiol 2004; 99: 165-172.
[137] Tang YL, Zhao Q, Zhang YC, Cheng L, Liu M, Shi J, Yang YZ, Pan C, Ge J, Phillips MI. Autologous mesenchymal stem cell transplantation induce VEGF and neovascularization in ischemic myocardium. Regul Pept 2004; 117: 3-10.
[138] Laguens R, Cabeza MP, Vera JG, Del Valle H, Lascano E, Negroni J, Werba P, Cuniberti L, Martinez V, Melo C, Papouchado M, OjedaR, Criscuolo M, Crottogini A. Entrance in mitosis of adult cardiomyocytes in ischemic pig hearts after plasmid-mediated rhVEGF165 gene transfer. Gene Ther 2002; 9: 1676-1681.
[139] Koury ST, Bondurant MC, Koury MJ. Localization of erythropoietin synthesizing cells in murine kidneys by in situ hybridization. Blood 1988; 71: 524-527.
[140] Vogt C, Pentz S, Rich IN. A role for the macrophage in normal hemopoiesis: III. In vitro and in vivo erythropoietin gene expression in macrophages detected by in situ hybridization. Exp Hematol 1989; 17: 391-397.
[141] Wu H, Lee SH, Gao J, Liu X, Iruela-Arispe ML. Inactivation of erythropoietin leads to defects in cardiac morphogenesis. Development 1999; 126: 3597-3605.
[142] Suzuki N, Ohneda O, Takahashi S, Higuchi M, Mukai HY, Nakahata T, Imagawa S, Yamamoto M. Erythroid-specific expression of the erythropoietin receptor rescued its null mutant mice from lethality. Blood 2002; 100: 2279-2288.
[143] Wald MR, Borda ES, Sterin-Borda L. Mitogenic effect of erythropoietin on neonatal rat cardiomyocytes: signal transduction pathways. J Cell Physiol 1996; 167: 461-468.
[144] Calvillo L, Latini R, Kajstura J, Leri A, Anversa P, Ghezzi P, Salio M, Cerami A, Brines M. Recombinant human erythropoietin protects the myocardium from ischemia-reperfusion injury and promotes beneficial remodeling. Proc Natl Acad Sci U S A 2003; 100: 4802-4806.
[145] Jaquet K, Krause K, Tawakol-Khodai M, Geidel S, Kuck KH. Erythropoietin and VEGF exhibit equal angiogenic potential. Microvasc Res 2002; 64: 326-333.
[146] Briest W, Homagk L, Baba HA, Deten A, Rassler B, Tannapfel A, Wagner KF, Wenger RH, Zimmer HG. Cardiac remodeling in erythropoietin-transgenic mice. Cell Physiol Biochem 2004; 14: 277-284.
[147] Wagner KF, Katschinski DM, Hasegawa J, Schumacher D, Meller B, Gembruch U, Schramm U, Jelkmann W, Gassmann M, Fandrey J. Chronic inborn erythrocytosis leads to cardiac dysfunction and premature death in mice overexpressing erythropoietin. Blood 2001; 97: 536-542.
[148] Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher AM, Dimmeler S. Erythropoietin is a potent physiologic stimulus for endothelial progeni tor cell mobilization. Blood 2003; 102: 1340-1346.