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Aging and Disease as Modifiers of Efficacy of Cell Therapy

Stefanie Dimmeler, Annarosa Leri

Abstract—Cell therapy is a promising option for treating ischemic diseases and heart failure. Adult stem and progenitor cells from various sources have experimentally been shown to augment the functional recovery after ischemia, and clinical trials have confirmed that autologous cell therapy using bone marrow—derived or circulating blood—derived progenitor cells is safe and provides beneficial effects. However, aging and risk factors for coronary artery disease affect the functional activity of the endogenous stem/progenitor cell pools, thereby at least partially limiting the therapeutic potential of the applied cells. In addition, age and disease affect the tissue environment, in which the cells are infused or injected. The present review article will summarize current evidence for cell impairment during aging and disease but also discuss novel approaches how to reverse the dysfunction of cells or to refresh the target tissue. Pretreatment of cells or the target tissue by small molecules, polymers, growth factors, or a combination thereof may provide useful approaches for enhancement of cell therapy for cardiovascular diseases. (Circ Res. 2008;102:1319-1330.)

Key Words: angiogenesis ■ diabetes ■ stem cells

Cell therapy using stem or progenitor cells is a promising option to improve the functional recovery after ischemia and restoration of heart function in patients with heart failure. Stem cells were considered to possess unlimited self-renewal capacity and to be able to replace themselves throughout the lifespan of the organism. However, it has recently been shown that a decrease in stem cell function plays a primary role in the pathogenesis of multiple diseases and tissue aging. Organ aging typically results in a marked decrease in the number of functionally competent stem cells dictated by forced entry of these cells in an irreversible quiescent state. DNA damage and apoptosis increase in aging stem cells, and these defects reduce further the pool of undifferentiated cells. In addition, risk factors for cardiovascular diseases such as diabetes and heart failure itself affect endogenous progenitor cells, thereby impairing endogenous repair and reducing the efficacy of patient-derived cells for therapeutic purposes. This review will summarize the impact of age and disease on bone marrow—derived and tissue-resident progenitor cells.
Impact of Disease and Aging on Bone marrow—Derived and Circulating Progenitor Cells

Cell therapy with bone marrow—derived stem/progenitor cells is a novel option for improving neovascularization and cardiac function in ischemic heart disease. The bone marrow contains different types of stem cells. Hematopoietic stem/progenitor cells (HPCs/HSCs), defined as CD34+ cells in humans or c-kit+ Sca-1+ lin− cells in mice, and mesenchymal stem cells (MSCs) have been successfully used to improve neovascularization and functional recovery in ischemic models. In addition, circulating hematopoietic or endothelial progenitor cells (EPCs; including proangiogenic cells), which can be mobilized from the bone marrow, were shown to give rise to new blood vessels and provide beneficial effects in vivo. Clinically, most studies so far used bone marrow—derived mononuclear cell preparation (BMC), which contains HSCs, EPCs, and—although to a very low extent—MSCs (for review see). In addition, ex vivo cultured EPCs, G-CSF—mobilized and purified circulating CD34+, and cultured MSCs were clinically applied. Although the mechanism of action may differ between HPCs, MSCs, and EPCs, the beneficial effects regarding functional recovery after ischemia were comparable in most if not all experimental studies. The initial preclinical studies have used human cells isolated from healthy young humans or young mice. When investigators started to isolate patient—derived cells, it was obvious that the number and functional activity of the cells are significantly impaired in comparison to healthy control. In addition to age, which is known to affect stem cells functions, risk factors for coronary artery disease (CAD) and heart failure diminish the capacity of the bone marrow—derived cell to contribute to functional repair (Figure 1). Although patients usually are exposed to more than one risk factor, the next paragraph will discuss the impact of the individual risk factors and disease entities on endogenous bone marrow—derived and circulating cells.

Impact of Cardiovascular Risk Factors

Diabetes is one of the key risk factors for CAD, and its prevalence has increased over the last few years. The analysis of cultured EPCs and CD34+KDR+ cells and their functions in clinical studies identified type II diabetes as a major determinant of impairment. Patients with type I and type II diabetes exhibit lower number of CD34+KDR+ EPCs or cultured EPCs and the reduced number of CD34+KDR+ cells was associated with the severity of diabetic vasculopathy.

These results were consistent with animal experiments with obese diabetic mice (Lerdb), in which the function of progenitor and proangiogenic cells were impaired. Of note, diabetes was not only associated with a reduction of cell numbers but led to a profound impairment of cell function such as reduced migration toward cytokines, reduced proliferation, and reduced ability of the cells to integrate into vascular networks in vitro. The impaired migratory response resembled the previously shown diminished response of diabetic monocytes to vascular endothelial growth factor (VEGF). Additionally, an increased sensitivity toward apoptosis or stress response of diabetic cells was described in some studies and high glucose concentrations induce senescence in cultured EPCs. If cells isolated from patients with diabetes were used to therapeutically enhance blood flow recovery in ischemia models, transplanted cells were significantly less effective compared to healthy control—derived cells. Likewise, the capacity of diabetic patient—derived cells to reendothelialize denuded arteries was impaired. In a skin wound assays, bone marrow—derived cells enriched for progenitor cells (lineage− fraction) isolated from obese diabetic mice even decreased vascularization. Such an inhibitory effect might be explained by the release of antiangiogenic factors (such as thrombospondin), which are increased in diabetic EPCs. Similar to the impaired function of circulating or bone marrow—derived cells by diabetes, other risk factors such as hypercholesterolemia and hypertension also were associated with reduced and dysfunctional circulating EPCs. In addition, circulating CD34+KDR+ or CD34+CD133+ KDR−CD45− cells were inversely correlated with smoking and this reduction was reversed by smoking cessation.

What are the mechanisms by which risk factors mediate their negative effect on progenitor cells? It appears that the signaling pathways mediating progenitor cell impairment are similar to the previously identified regulators of endothelial cell function and atherosclerosis and include a dysregulation of nitric oxide (NO) and reactive oxygen species (ROS). A diabetic environment or high glucose exposure in vitro is associated with reduced nitric oxide (NO) bioavailability in cultured EPCs, and plasma levels of endogenous NO synthase inhibitors (asymmetrical dimethyl-l-arginine) are associated with clinically reduced EPC numbers. In exper-
imental studies, endothelial NO synthase (eNOS)-derived NO was shown to be essential for basal, VEGF-, and SDF-1–induced migration of EPCs or bone marrow–derived progenitor cells, and eNOS+/− progenitor cells showed a reduced homing capacity in ischemia models in vivo.30,31 The underlying mechanisms mediating the NO effects, eg, on chemokines or integrin expression or signaling, need to be further defined. Despite various studies supporting an important role of eNOS for progenitor cell mobilization and function,30,32,33 under certain conditions, eNOS uncoupling may lead to an increased ROS production.16 To what extent the redox balance in stem cells (as opposed to cultured EPCs or crude bone marrow homogenates used in the study16) favors such uncoupling processes is unclear and deserves further studies.

The impact of reactive oxygen species (ROS) appears more complex. Several studies demonstrated that ROS production is increased in patient-derived cells and that ROS production is associated with functional impairment of progenitor cells.34,35 A putative role of ROS for stem/progenitor cell dysfunction is supported by studies demonstrating that chemically-induced oxidative stress limits the life span of HSCs.36 However, other studies failed to reverse glucose-induced early and outgrowing EPC dysfunction by antioxidant treatment.37 It might be that the differential response is caused by the use of cells equipped with a different antioxidative defense system. For example, cultured early EPCs were shown to express high levels of Mn-superoxide dismutase and catalase,38,39 which might render this specific subtype of cultured cell less prone to ROS-induced damage or dysfunction. Likewise, the analysis of the transcriptome of stem cells showed an enrichment of genes promoting resistance to environmental stresses.40 Moreover, physiological concentrations of ROS might be critically involved in migration and mobilization of progenitor cells, thereby, ROS might interfere with stem cell homeostasis on different levels resulting in opposing effects. Depending on the conditions (eg, diminished antioxidative defense, excessive prolonged oxidative stress) the positive effect of endogenous ROS may be overridden by the harmful effects.41

Risk factors, and particularly diabetes, additionally activate proinflammatory protein kinases such as the mitogen activated protein (MAP) kinase p38, thereby reducing proliferation and differentiation of cultured EPCs.42 Activation of p38 might also be a consequence of ROS production,43 although this link has not been experimentally demonstrated under diabetic conditions.

Finally, risk factors might profoundly affect the therapeutic benefit of cells by interfering with homing functions of progenitor cells. Only a few studies so far directly addressed the impact of risk factors on the complex set of processes mediating progenitor cell homing, which involve chemokine-induced attraction of cells and protease-mediated invasion and matrix degradation. Of the multiple steps involved in homing and recruitment, the interaction of the ischemia-induced cytokine stromal-derived factor-1 (SDF-1) with its receptor CXCR4, which is expressed on HSCs and EPCs, is crucial for cell-recruitment.42,43 This critical pathway appears to be impaired in EPCs cultured from coronary artery disease patients, which do not adequately responds to activation of the CXCR4 receptor.44 Reduction of CXCR4 expression in heterozygote mice to 50% reduced basal and SDF-1–induced migration and abolished in vivo homing.44 Therefore, one may speculate that the defective CXCR4 signaling in patient-derived cells contributes to the migration and homing defect, which is associated with a reduced clinical benefit of cell therapy in pilot trials.45

In summary, ample experimental studies and clinical observations provide evidence that cardiovascular risk factors interfere with circulating progenitor and proangiogenic cells. An important question is whether the impairment of circulating progenitor cells and proangiogenic cells in patients with high risk factor load is caused by a depletion of stem cell reserves and stem cell exhaustion in the bone marrow or might be attributable to signaling defects and increased apoptosis of circulating cells. Limited studies are available to answer this question, particularly because in the clinical setting bone marrow–derived cells are not as easily accessible as circulating cells and classical stem cell assays (eg, repopulation activity) with cells isolated from high-risk patients have not been performed so far.

Impact of Age and Telomere Shortening

In healthy individuals and patients with coronary artery disease, age is associated with a reduced number and function of cultured EPCs, circulating CD34+/KDR+/cells, or CD133+/cells and of granulocyte macrophage colony forming units (GM-CFU) in the bone marrow.7,46,47 First evidence that age directly affects cell-mediated improvement of neovascularization was provided by Edelberg et al, who demonstrated that young, but not old, bone marrow cells incorporated into the neovascularature and restored cardiac angiogenic functions.48 On a molecular level aging is linked to a reduction of telomere length. The proliferative history of a cell is written on telomeres: telomere erosion reflects the number of past divisions experienced by a cell and its proliferative potential.49 In addition, telomere erosion may contribute to telomere shortening. When long telomeres protect chromosomal ends, cells can undergo repeated cell divisions. Conversely, telomere shortening beyond a critical length leads to genomic instability, DNA damage, p53 activation, and ultimately cell cycle arrest.50 Telomere shortening as it occurs with aging is associated with replicative exhaustion and reduced repopulation of HSC.51 Aging also is associated with a reduction of telomere length in circulating but also in bone marrow–derived cells.52 Interestingly, telomere length in circulating blood leukocytes and bone marrow–derived cells was not only associated with age but also reduced in patients with coronary artery disease or heart failure indicating that cardiovascular disease promotes telomere shortening.53,54 While the limited number of cells available in the patient study precludes any information on the impact of age on telomere length of freshly isolated specific subpopulations such as CD34+ cells (or other stem cell populations), the reduction of telomere length in patient-derived cells was associated with a reduced ex vivo migratory response of the total BMC toward the chemokines VEGF and SDF-1, indicating that age-associated telomere reduction may contribute to impaired functionality of the cells.52 In addition, a reduced telomere
Impact of Disease and Aging on Tissue-Resident Stem Cells

Various experimental studies document that tissue-resident stem or progenitor cells improve recovery after ischemia. The recognition that the adult heart possesses a pool of resident cardiac progenitor cells (CPCs), which are self-renewing, clonogenic, and multipotent, has dictated a new area of research offering the potential to harvest cells, which are primed to acquire a cardiac phenotype and, therefore, might be optimally suited for cardiac repair. Several different populations have been identified and characterized: c-Kit+ cells, Sca-1+ cells, side population cells, and cells expressing the protein Islet-1. Whereas c-Kit+ cells, Sca-1+ cells, and cardiac SP cells are isolated from adult hearts, cells expressing Islet-1 so far only have been detected in the postnatal stage. Whether c-Kit+, Sca-1+, and cardiac SP cells comprise three different cell populations is not entirely resolved. At least Sca-1+ cells contain a fraction of SP cells and among cardiac SP cells, the greatest potential for cardiomyogenic differentiation is restricted to cells positive for Sca-1 expression (but negative for CD31), indicating that the two cell populations may share some common features.

Cardiac stem cells also were obtained by growing self-adherent clusters (termed “cardiospheres”) from subcultures of murine or human biopsy specimens. Others have generated cardiac SP-cell–derived cardiospheres by adapting a method used for creating neurospheres and claimed that cardiac neural crest cells contribute to cardiac SP cells. Cardiosphere-derived cardiac stem cells as well as c-Kit+ cardiac stem cells are capable of long-term self-renewal and can differentiate into the major specialized cell types of the heart: myocytes and vascular cells (i.e., cells with endothelial or smooth muscle markers). So far, the origin and the mechanisms maintaining the cardiac stem cell pool are unclear. Whereas two recent studies suggested that c-Kit+ and cardiac SP cells may derive from the bone marrow, these studies cannot exclude that specific subpopulations of cardiac stem cells origin from distinct sources and may represent remnants from embryonic development in selected niches within the heart.

Adipose tissue is an additional source of distinct subsets of stem/progenitor cells potentially useful for cardiac repair and neovascularization improvement. Both, mesenchymal stem cells and endothelial progenitor cells were isolated after enzymatic digestion of adipose tissue and showed beneficial effects in experimental studies. The therapeutic benefit of adipose-tissue derived cells is currently studied in two clinical trials.

Although the relatively easy accessibility of circulating progenitor cells and the use of bone marrow–derived cells in clinical studies allowed getting first insights into the number and function of cells in patients with CAD or heart failure, the knowledge on the influence of disease and aging on CPC in humans is limited and the regulation of adipose tissue-derived cells during disease states is unknown. Therefore, this section will summarize the influence of heart failure, diabetes, and aging on CPC homeostasis and function.
Impact of Disease
Experimental studies demonstrated that the CPC pool size is expanded acutely after infarction. However, this growth response is attenuated in chronic heart failure. 

Myocyte division is markedly reduced in prolonged ischemic and dilated cardiomyopathy, suggesting that a decrease in number and growth of CPCs underlies the attenuation in cell multiplication in end-stage heart failure. Telomerase activity in CPCs is decreased and cell proliferation is impaired by severe telomeric shortening and irreversible growth arrest. This replicative defect coupled with increased CPC apoptosis results in a decline of the pool of functional CPCs so that the formation of myocytes and coronary vessels can no longer counteract the chronic loss of parenchymal cells and vascular structures. This negative balance between myocardial regeneration and death may ultimately lead to progressive chamber dilation and deterioration of ventricular performance.

However, even under extreme conditions of depressed ventricular function, a pool of cycling and telomerase-competent CPCs can be found and two independent laboratories have demonstrated that CPCs can be isolated from small samples of human myocardium and expanded in vitro with the purpose to be administrated back to the same patient. Although the growth curves of cardiospheres isolated from right ventricular endomyocardial biopsies of 59 transplanted (age: 53.6 years with left ventricular ejection fraction [EF] 63.9%) and 11 nontransplanted patients (age: 47; EF: 36.9%) showed a considerable heterogeneity, this study did not identify a major independent determinant of cell yield after culture. These data clearly indicate that cardiospheres can be obtained from patients with reduced ejection fraction; however, a detailed comparison of the functional capacities of the isolated cells and a comparison with cardiospheres derived from healthy controls has not yet been published.

In a manner similar to ischemic cardiomyopathy, diabetes is coupled with a marked reduction of the pool size of CPCs. A typical feature of the diabetic heart is the development of a myopathy that deteriorates with time, independently from intercurrent vascular manifestations. This myopathy presents itself as ventricular dilation, relative wall thinning, and impaired diastolic and systolic function. The imbalance between cardiac cell death and cell regeneration characterizes the diabetic heart. However, the death of mature myocytes, SMCs, and ECs is only a secondary phenomenon; the premature aging and death of CPCs precedes the progression of the diabetic myopathy. The accumulation of old myocytes is mediated by a dramatic loss of CPCs that markedly attenuates the formation and efficient turnover of parenchymal cells. Importantly, the adaptor protein p66shc plays a major role in the effects of diabetes on the heart. In fact, ablation of p66shc has remarkable beneficial consequences on the viability and function of CPCs, positively interfering with death stimuli and inhibition of CPC growth and differentiation. CPC division and myocyte generation are potentiated by the absence of p66shc, which opposes the development of a compensated diabetic heart. Expansion of the CPC pool and myocyte progenitor precursors is combined with the preservation of cardiac performance, suggesting that an intact stem cell compartment can counteract the impact of uncontrolled diabetes on the myocardium. This observation may have important implications for the treatment of patients affected by diabetes and cardiac diseases. The deletion of p66shc may offer an early cardiac protection that could be exploited clinically. Diabetes, thus, negatively affects the growth reserve of the heart so that the enhanced cell death cannot be opposed by repopulating cells and preservation of the architecture and function of the myocardium.

Impact of Aging
In old rats, chronological age leads to telomeric shortening in CPCs, which by necessity generate a differentiated progeny that rapidly acquires the senescent phenotype. The daughter cells inherit the shortened telomeres of the maternal CPCs and, after a few rounds of division, express the senescence-associated protein p16INK4a. The pool of old cardiomyocytes progressively decreases and ventricular function is impaired. However, telomerase competent CPCs with long telomeres are present in the regions of storage in the atria and apex and these cells, after activation by growth factors, migrate to areas of damage where they create a population of young myocytes reversing to some extent the aging myopathy structurally and functionally. The senescent heart phenotype is partially corrected and the improvement in cardiac hemodynamics results in prolongation of maximum lifespan in the rat model.

Mechanism of CPC Impairment During Disease and Aging
The loss of CPC function with aging is mediated partly by an imbalance between factors promoting growth, migration, and survival, and factors enhancing oxidative stress, telomere attrition, and death. Three growth-factor receptor systems appear to play a major role in the development of CPC senescence and myocardial aging: IGF-1–IGF-1R, HGF-c-Met, and the rennin angiotensin system (RAS). In the heart, the IGF-1–IGF-1R induces CPC division, upregulates telomerase activity, hinders replicative senescence, and preserves the pool of functionally-competent CPCs. The expression of IGF-1R and the synthesis of IGF-1 are attenuated in aging CPCs and these negative variables diminish the ability of IGF-1 to activate cell growth and interfere with oxidative damage and telomeric shortening. Additionally, the expression and secretion of HGF in CPCs decreases as a function of age and this modification has a major impact on the migration of CPCs and thereby on the ability of these cells to translocate spontaneously to areas of damage and promote cardiac repair. Defects in these two autocrine-paracrine effector pathways of CPCs may have profound physiological consequences and may account for the chronological increase in myocyte death, myocardial scarring, and depressed performance of the aging heart.

The documentation that the various components of RAS are present in CPCs and the formation of Ang II is enhanced in old cells provides evidence in favor of the role of this octapeptide in CPC senescence and death. Ang II may be a significant contributor of the age-dependent accumulation of oxidative damage in the heart. Inhibition of Ang II function positively interferes with heart failure and prolongs...
life in humans. Ang II generates reactive oxygen species (ROS) and sustained oxidative stress triggers telomeric shortening and uncapping. Conversely, IGF-1 interferes with the generation of ROS, decreases oxidative damage in the myocardium with age, and can repair oxidative DNA damage by homologous recombination. Collectively, these findings suggest that cardiac aging is associated with a dysfunction of the endogenous stem cell pool that is dictated partly by the imbalance between RAS and IGF-1/HGF.

Effort has been made to identify the etiology of CPC death with diabetes. Diabetes promotes the generation of reactive oxygen species (ROS) and DNA damage. Oxidative stress and p53 activation have more dramatic effects on cells capable of reentering the cell cycle and dividing than cells which are permanently withdrawn from the cell cycle. The DNA damage mediated by oxidative stress rapidly initiates the apoptotic cell death pathway in CPCs while it accumulates with time in myocytes. CPCs undergo apoptosis and experience cell necrosis only occasionally. Conversely, myocyte apoptosis is followed by cell necrosis and foci of myocardial scarring. The differential response of CPCs and myocytes to diabetes results over a short period in a marked reduction of the pool size of CPCs that is several fold higher than that of myocytes. As discussed above, the adaptor protein p66Shc plays a major role in the effects of diabetes on the heart. Ablation of p66Shc has remarkable beneficial consequences on the viability and function of CPCs positively interfering with death stimuli and inhibition of CPC growth and differentiation. CPC division and myocyte generation are potentiated by the absence of p66Shc opposing the development of the diabetic myopathy. Expansion of the CPC pool and myocyte progenitors-precursors is combined with the preservation of cardiac performance, further suggesting that an intact stem cell compartment can counteract the impact of uncontrolled diabetes on the myocardium.

It is likely that during aging and chronic diseases, stem cells tend to become quiescent. While quiescence in young active progenitor cells is modulated by p21Cip191 in old/diseased stem cells irreversible growth arrest is regulated by p16Ink4a. Loss of telomerase, critical telomere shortening, and increased nuclear expression of p53 and p16Ink4a may all occur resulting in loss of growth reserve.2 Early stem cell depletion may induce premature aging while replenishment of stem cells in depleted organs may reverse aging and disease promoting positive remodeling and recovery of function.

**Influence of Disease and Aging on the Target Tissue**

When transplanting cells in patients, one also has to take into account that the environment in which the cells are infused or injected might be affected, thereby, modulating homing, incorporation, and differentiation cues. Little is known about the influence of disease and aging on homing and functional integration requiring the complex interaction of the injected cells with the environment. Such environmental changes could reduce the ability of transplanted cells to contribute to functional repair on various levels. Several studies suggested the homing signals might be impaired in disease. As discussed above, the cytokine SDF-1 is essential for the homing of endogenous and intravascularly infused progenitor cells. However, SDF-1 mRNA and SDF-1 positive cells were significantly reduced in wound tissue of diabetic mice. These experimental studies are supported by findings in patients with peripheral artery disease, where the expression of VEGF, SDF-1, and CXCR4 in human limb muscle was significantly suppressed even below the levels detected in controls suggesting a lack of recruitment signals in these patients.

Age per se also interferes with the environment and also decreased hypoxia-inducible factor 1a leading to diminished expression SDF-1. Interestingly, a reduction of telomere length in Terc-deficient mice was associated with a dysfunction of the bone marrow stem cell niche as evidenced by limited function and engraftment of transplanted wild-type HSC to the bone marrow of Terc-deficient mice. A change in the cytokine milieu (increased G-CSF levels) was observed in the mutant mice indicating that a disturbed cytokine milieu might have contributed to the dysfunction. Whether a similar effect can be seen in other organs is unknown, however it is known that aging and disease influence the cytokine profile in the heart. Despite the limited knowledge with this respect, it is conceivable that the composition of an established healed scar or fibrotic tissue in an old and chronically ill patient provides an entirely different milieu for the transplanted cells compared to the acute injury models (in young and healthy animals) usually used in the experimental studies.

**Therapeutic Strategies to Interfere With Disease and Age Related Dysfunction**

The demonstration of impaired cell function and environment in disease and aging demands the development of strategies to overcome these limitations. The use of heterologous cells might be an option to compensate for the reduction in cell functions; however, this strategy also comprises additional risks. Particularly, when heterologous cells are expected to functionality differentiate in cardiovascular cells, activation of the immune response or even cell rejection might occur proposing a high risk for the treated patients. The pretreatment of the patients own cells to reactivate their functions or direct their differentiation might be an alternative to improve the efficiency of cell therapy in the chronically ill. Second, the treatment of the target tissue in order to provide the cytokines and chemoattractant factors to stimulate incorporation of the transplanted cells or to reactivate endogenous repair might be considered.

**Preactivation of Cells**

To compensate for the risk factor-induced cell impairment, several studies used pharmacological approaches to interfere with the elicitor. Of note, the need to improve survival and incorporation is not limited to the use of patient-derived cells, and various approaches have been experimentally used to increase survival of cells derived from healthy controls, murine cells, and embryonic stem cells (for review see97). For example, overexpression of prosurvival kinases such as Akt or the telomerase subunit TERT, to compensate for age-associated telomere length reduction, significantly improved

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The functional recovery after AMI mediated by transplantation of MSC- and EPC-mediated limb perfusion, respectively.98,99 Lipid lowering HMG-CoA reductase inhibitors (statins) were first reported to increase the number of EPCs in patients and in experimental models.26,33,100,101 In addition to the systemic effects, statins directly augmented the migratory capacity of EPCs in vitro and prestimulation of EPCs with statins increased their neovascularization-promoting effect. The mechanisms by which statins affect the in vitro functions of EPCs involve the activation of the PI3-kinase and Akt and subsequent activation of the eNOS.33,100,101 NO plays a crucial role in progenitor cell function, and the expression of eNOS is required for bone marrow–derived mobilization of EPCs.30,32,33,102 Because NO bioavailability is systematically deteriorated in patients with coronary artery disease, an increased NO synthesis by statins may function of NO-deprived stem/progenitor cells. The nitric oxide system also was activated by compounds, which transcriptionally enhance eNOS expression in hematopoietic and endothelial progenitor cells.31 Specifically, the pretreatment of patient-derived EPCs or BMCs with eNOS enhancers for 24 hours significantly enhanced the capacity of the intravenously infused cells to restored neovascularization, augmented the exercise capacity in a hind limb ischemia model, and improved recovery after acute myocardial infarction.31 Other possibilities to augment eNOS expression and activity include the use of PPARγ agonists or systemic application of estrogen.102–104 Verma et al demonstrated that PPARγ agonists prevented the suppression of EPC function by C-reactive protein in an NO-dependent manner in vitro.105 Consistently, the PPARγ agonist rosiglitazone recently was shown to restore dysfunctional EPC from diabetic patients and improve the endothelial regenerating activities of infused progenitor cells.24 The diabetic impairment of NO-mediated progenitor cell functions leading to wound healing defects was rescued by hyperoxia and SDF-1 application.91 In diabetic patient–derived cells the blockade of kinases, which are upregulated during disease such as the MAP-kinase p38, also might be an option to augment cellular functions. Pharmacological inhibitors of p38 indeed improved peripheral blood–derived cells isolated from patients with diabetes and cardiovascular diseases.22

In addition to counteracting risk factor–induced impairment and augmenting survival of injected cells, short term activation of receptors and signals involved in homing might be considered to improve the modest engraftment of cells. Because the SDF-1/CXCR4 axis is playing a crucial role in progenitor cell homing, several attempts to enhance this signaling cascade. In cells, which are characterized by low to absent expression of CXCR4 such as mesenchymal stem cells, overexpression of CXCR4 enhanced the in vivo engraftment to ischemic tissue.105 Activation of CXCR4 was used as an alternative approach in several cell types. CXCR4 was transactivated by sphingosin-1–phosphate (S1P), leading to enhanced EPC homing of patient-derived proangiogenic cells and improved recovery of blood flow in hind limb ischemia.23 Surprisingly, also prestimulation of the CXCR4 receptor directly by SDF-1 increased transendothelial migration of mesoangioblasts and stimulated the colonization and muscle fiber repair.106 Finally, integrin-dependent homing functions can be modulated by inflammatory cytokines such as the high mobility group box protein 1 (HMGB-1)107 or by intracellular activation of integrin signaling.108 For example, pharmacological activation of Epac1, a nucleotide exchange protein for Rap1, induces integrin polarization and activity and improves the adhesive and migratory capacity of distinct progenitor cell populations including human EPCs, CD34+ HPCs, and MSCs.108 Others identified an additional interesting ischemia-induced player, soluble E-selectin (s-E-selectin), which stimulates the homing of progenitor cells,109 whereas overexpression of the membrane form of E-selectin was shown to enhance the functional activity of endothelial progenitor cells.110 In summary, various strategies might be considered to enhance the engraftment and survival of cells applied to the heart leading to a second generation of cell therapy products. Which of the strategies will be chosen and will provide the best benefit, likely depends on the cell type and the delivery mode of the cells (Figure 2).

**Treatment of the Target Tissue to Improve Homing and Engraftment**

The “refreshment” of the target tissue might become an important add-on to enhance recruitment and functional incorporation of any transplanted cell to the diseased, aged and chronically ill patient. Injection of cytokines might be...
used to attract progenitor cells in the absence of necrosis or acute ischemia (Table). Indeed, transplantation of syngeneic cardiac fibroblasts stably transfected to express SDF-1 induced homing of c-kit+ cells to the myocardium.43 An elegant approach to further enhance SDF-1 effects was used by Seger et al who generated a stabilized SDF-1 mutant.111 SDF-1 can be cleaved by exopeptidases and matrix metalloproteinas-2, thereby generating an inactive and even harmful protein. By mutating the SDF-1 cleavage motif, the authors generated a cleavage resistant SDF-1 molecule and demonstrated that nanofiber delivery of the protease-resistant SDF-1 more efficiently improved cell recruitment and functional recovery after acute myocardial infarction compared to wild type SDF-1. Similarly, local myocardial delivery of insulin-like growth factor 1 (IGF-1) with biotinylated peptide nanofibers improved the effect of cell therapy with intramuscularly injected neonatal rat cardiomyocytes after myocardial infarction.80 Likewise, the IGF binding protein 3 either locally injected or when overexpressed in the injected cells, promotes proper vascular repair after hyperoxic insult mediated by hematopoietic and endothelial progenitor cells.112 Moreover, local injection of HMGB-1 enhanced the regeneration of infarcted myocardium by endogenous cardiac stem cells113 and attracted systemically injected cells to muscle tissue.114

In addition to direct delivery of growth factors, it might be conceivable to activate the tissue by inducing the endogenous expression of cytokines. Indeed, activation of the tissue by low energy shock wave application stimulated the expression of SDF-1 and VEGF within the target tissue and promoted homing of intravenously infused EPC in uninjured and chronically ischemic rats.115

**Table. Strategies to Increase Progenitor Cell Function, Survival, and Homing**

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<th>Genes</th>
<th>Growth Factors/Cytokines</th>
<th>Small Molecules</th>
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<tr>
<td>Akt*</td>
<td>HMGB-1†</td>
<td>Statins*#</td>
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<td>TERT*</td>
<td>Growth hormone#</td>
<td>PPARγ agonists*#</td>
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<td>HGF†</td>
<td>Nitric oxide enhancers*</td>
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<td>GS3K-inhibitor*</td>
<td>IGF-1 (plus nanofibers)†</td>
<td>p38 inhibitors*</td>
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<td>IGF-1†</td>
<td>SDF-1†</td>
<td>EPAC activators*</td>
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<td>IGF binding protein-3†</td>
<td>Soluble E-selectin†</td>
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<td>VEGF*</td>
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*Treatment of the cells; #systemic treatment; †treatment of the target tissue.

Modulation of Common Endogenous Modifiers of Stem Cell Exhaustion and Organism Aging

Changing the environment may not only be useful to attract transplanted cells, but also might be an attractive approach to increase endogenous repair capacity. In fact, proliferative defects of old or diseased progenitor cells depend not only on cell-intrinsic mechanisms but also on structural alterations of the niches that host the undifferentiated cell compartment.96,116,117 This aspect has been studied in the skeletal muscle in which aged niches oppose the regenerative response of endogenous satellite cells and transplanted embryonic stem cells.116,118 It is reasonable to assume that similar mechanisms are operative in the myocardium. Cardiac aging and diseases may have inhibitory effects on stem cell activation, migration, and growth. Prevention of cell death and induction of cell replication are the ultimate goal of cell therapy; the former attenuates the extent of injury and the latter determines the degree of structural and functional recovery. This goal may be achieved not only by correcting the intrinsic defects of stem cells but also modifying the environmental niche.

Attempts have been made experimentally to document that changes of the cardiac milieu have critical effects on the regenerative ability of resident CPCs. Myocyte-restricted overexpression of IGF-1 is associated with delayed aging of CPCs and myocytes and retarded onset of ventricular dysfunction.79 IGF-1 promotes myocyte formation and positively interferes with the effects of myocardial infarction, chronic coronary artery constriction, and diabetes on ventricular remodeling and heart failure. Similarly, a skeletal muscle specific IGF-1 isoform counteracts the decline in mass and functional performance in senescent IGF-1 transgenic mice and mdx mice suffering from Duchenne muscular dystrophy.119,120 Growth hormone-induced increase in IGF-1 additionally improved age-associated dysfunction of EPC in experimental models and humans.121 Paradoxically, studies in nematodes or fruitflies demonstrated opposing effects of IGF-1 on aging and life span.122 The mechanisms underlying these controversial IGF-1 activities have not been clarified.

Another cytokine linked to cardiac repair and aging is the platelet-derived growth factor (PDGF). PDGF is downregulated during aging, and its supplementation reversed the senescent predisposition to increased cardiac injury.123 Interestingly, a reduced expression of PDGF was detected in aged Oct3/4+ bone marrow–derived stem cells, and the age-dependent downregulation of PDGF was associated with an impaired cardiac differentiation capacity of the cells.124 In addition to insulin/IGF-1 and PDGF, an important modulator of lifespan and aging is the transmembrane protein Klotho.125 The extracellular domain of Klotho circulates in the blood,126 suggesting that this protein may function as an antiaging hormone possibly influencing the regenerative response of tissue-resident stem cells in multiple organs. Klotho mutant mice have a very short lifespan and display a variety of aging-related phenotypes in multiple organs, including atherosclerosis, decreased fertility, impaired angiogenesis, and skin atrophy.127 In contrast, the overexpression of Klotho in mice extends lifespan.125 This effect was initially linked to the attenuation of insulin/IGF-1—signaling,128 but the consequences of Klotho overexpression are more complex. Klotho increases the cellular resistance to oxidative stress by upregulation of manganese superoxide dismutase and is an essential cofactor of fibroblast growth factor signaling.128 It is difficult to establish which one of these effects is the determining variable of prolonged lifespan. A human homologue of Klotho has been identified, and a functional variant of Klotho, termed KL-VS, is associated with human aging, reduced longevity, and early-onset of coronary artery disease.129 In a recent study, Klotho deficiency was found to be
coupled with enhanced Wnt signaling.\textsuperscript{130} Klotho binds to several members of the Wnt family of proteins and is a powerful repressor of the Wnt pathway. This work has revealed a critical interaction among secreted factors, intracellular signaling pathways, and stem cell function. In fact, chronic Wnt stimulation dictated by the absence of Klotho results in precocious senescence of skin stem cells and exhaustion of the long-term repopulating pool of hematopoietic stem cells in the bone marrow.\textsuperscript{130,131} Of note, the effects of Wnts are complex, and short term activation might be useful to stimulate proliferation or modulate differentiation cues.\textsuperscript{132}

In summary, the mechanisms underlying organ and organism aging and the impact of disease on organ functions are complex and only in part understood. Caution has to be exercised in the translation of results in simple postmitotic organisms to large mammals and particularly humans. This is because the life and death of most somatic organs in mammals is regulated by a stem cell compartment, which plays a critical role in aging and in the response of organs to disease. The complex relationship between stem cells and their surrounding tissue has to be considered as a critical determinant for the success of cell therapy. As such the impact of disease and age on endogenous stem and progenitor cells and on the environment may limit the benefit of cell therapy of the chronically ill patients; however, this also opens a new horizon for therapeutic strategies to counteract the dysregulated cell intrinsic and extrinsic signaling pathways.

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