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Stem Cells and the Regeneration of the Aging Cardiovascular System

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This Review is part of a thematic series on the **Biological Role of Senescence in Cardiovascular Disease**, which includes the following articles:

Telomere Biology and Cardiovascular Disease
Vascular Cell Senescence: Contribution to Atherosclerosis

Stem Cells and the Regeneration of the Aging Cardiovascular System

Mechanisms of Cardiovascular Disease in Accelerated Aging Syndromes
Mechanisms Underlying Caloric Restriction, Lipid Metabolism, and Life Span Regulation

Issei Komuro, Guest Editor

Stem Cells and the Regeneration of the Aging Cardiovascular System

Victoria L.T. Ballard, Jay M. Edelberg

Abstract—It is well established that cardiovascular repair mechanisms become progressively impaired with age and that advanced age is itself a significant risk factor for cardiovascular disease. Although therapeutic developments have improved the prognosis for those with cardiovascular disease, mortality rates have nevertheless remained virtually unchanged in the last twenty years. Clearly, there is a need for alternative strategies for the treatment of cardiovascular disease. In recent years, the idea that the heart is capable of regeneration has raised the possibility that cell-based therapies may provide such an alternative to conventional treatments. Cells that have the potential to generate cardiomyocytes and vascular cells have been identified in both the adult heart and peripheral tissues, and in vivo experiments suggest that these cardiovascular stem cells and cardiovascular progenitor cells, including endothelial progenitor cells, are capable of replacing damaged myocardium and vascular tissues. Despite these findings, the endogenous actions of cardiovascular stem cells and cardiovascular progenitor cells appear to be insufficient to protect against cardiovascular disease in older individuals. Because recent evidence suggests that cardiovascular stem cells and cardiovascular progenitor cells are subject to age-associated changes that impair their function, these changes may contribute to the dysregulation of endogenous cardiovascular repair mechanisms in the aging heart and vasculature. Here we present the evidence for the impact of aging on cardiovascular stem cell/cardiovascular progenitor cell function and its potential importance in the increased severity of cardiovascular pathophysiology observed in the geriatric population. **(*Circ Res*. 2007;100:1116-1127.)**

Key Words: cardiac stem cell ■ endothelial progenitor cell ■ regeneration ■ aging

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States.[1] CVD risk increases with age and, in older patients, is associated with increased rates of complications and poorer clinical outcomes. As a consequence, CVD is predominant within the elderly population, with approximately 85% of all deaths attributable to CVD in the United States occurring in individuals more than 65 years of age.[1] Age-associated changes in many aspects of cardiovascular function appear to contribute to this increased risk. These include: (1) impairment in the regulation of vascular tone that is attributable, for example, to decreased NO production and increased levels of angioten-

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sins and endothelin[2,3]; (2) increased coagulation activity and decreased fibrinolytic capacity, leading to increased risk of coronary thrombosis[4,5]; (3) dysregulated angiogenic repair mechanisms that are important for restoration of blood flow after ischemic injury[6]; and (4) a decrease in the number and function of circulating endothelial progenitor cells (EPCs), which are known to contribute to neovascularization.[7–9] Furthermore, cellular senescence, resulting from the accumulation of oxidative damage, telomere shortening, and the loss of replicative ability in vascular cells[10] also contributes to the progressive inability of the heart and vasculature to repair themselves after injury.

Many of these changes occur within the cells of the heart and vasculature themselves. However, systemic changes associated with aging, including decreased hormone levels and added complications attributable to the presence of other age-associated pathologies, such as diabetes and hypertension, also play a major role in the increased risk of CVD in older individuals. Current therapies, including treatments for acute coronary syndromes such as percutaneous coronary interventions and anticoagulants, and preventative strategies such as statins and angiotensin converting enzyme inhibitors, have improved the quality of life for those with CVD. However, there has been little change in overall deaths from CVD over the last 2 decades,[11] demonstrating the need for improved therapeutic strategies, particularly those that are effective in treating the aging population. Recently, there has been intense focus on the use of cardiac progenitor cells (CPCs) to replace damaged myocardium and/or to initiate endogenous mechanisms of cardiovascular repair. The promise of cell-based therapies to restore damaged tissues in the heart and associated vasculature has led to the initiation of clinical trials to examine the effectiveness of these cells in improving cardiac function after ischemic injury.[12–21] Although the use of CPCs and cardiac stem cells (CSCs) as a potential new therapy for the treatment of CVD is thus receiving much attention, an understanding of the potential changes in these cells with aging may improve their therapeutic utility for older individuals, who may have the greatest need for robust treatment. Moreover, as a number of studies suggest that there is a progressive dysregulation of CSC and CPC generation and function with increasing age, strategies based on countering these changes offer the promise of reducing the increased severity of CVD in older persons.

Identification of Stem and Progenitor Cells for Cardiovascular Regeneration

Cardiac Stem Cells

The identification of stem- and progenitor-like cells that appear to have the capacity to differentiate into myocytes and vascular cells represents a potentially useful approach to the development of novel therapeutics for cardiovascular repair. The presence of stem cells resident in the heart itself was first reported by Beltrami et al in 2003. This group identified c-kit$^+$ cells located in the myocardium that were positive for mitotic markers and transcription factors indicative of cardiomyogenic differentiation, including GATA4 and Nkx2.5.[22] In vitro, individual c-kit$^+$ cells isolated from the adult heart have the capacity to differentiate into myocardial, endothelial, and smooth muscle cell lineages and are also capable of self-renewal. Most strikingly, transplantation of these cells into the hearts of syngeneic rats post–myocardial infarction reduced the extent of myocardial damage compared with controls, and the transplanted cells were able to give rise to myocyte, endothelial, and smooth muscle cell lineages. Shortly after this discovery, Oh et al described the isolation of a subpopulation of Sca-1$^+$, c-kit$^-$ cells from the heart that express a range of markers typical of a cardiac phenotype. When transplanted intravenously into mice with myocardial infarctions, these cells were able to home to the site of injury and appeared to have the capacity to differentiate into cardiomyocytes.[23] Together, the data from both groups provided compelling evidence that the heart contains resident CSCs/CPCs and is therefore capable of regeneration.

Since the publication of these studies, a number of other groups have reported the identification of resident CSCs capable of differentiation into cardiomyocytes and vascular cells and, in some cases, also have shown that they can improve cardiac function after myocardial infarction.[24–26] However, a number of important questions still remain regarding the basic biology of CSCs. For example, each group that has identified a CSC population has used different phenotypic markers to isolate these cells. This may be attributable to the fact that several subpopulations of cells in the adult heart have the capacity to give rise to the major cardiac cell lineages or that CSCs undergo phenotypic changes in response to environmental influences. Because of this heterogeneity, a single molecular phenotype of the CSC has not been defined. It is also unclear whether the major contribution of these CSCs to the repair of damaged myocardium is via the physical replacement of myocytes and vascular cells or through the secretion of growth factors and cytokines that enhance endogenous cardiovascular repair mechanisms.[27–30] Indeed, a number of studies have suggested that CSC differentiation does not occur in the heart, and transplanted cells instead appear to take on cardiac phenotypes simply through fusion with existing cells (see further discussion below). Lastly, there is currently no evidence that CSCs contribute to cardiac repair via differentiation in the absence of experimental intervention; therefore, their role in endogenous mechanisms of cardiac regeneration remains unknown.

Bone Marrow–Derived Cardiac Stem and Progenitor Cells

Besides cardiac stem cells located in the heart itself, a number of extracardiac cell populations are also capable of giving rise to cardiac cell types. The most widely studied source of these CSCs/CPCs is the bone marrow. The ability of the bone marrow to give rise to endothelial cells was first reported by Shi et al.[31] This study was based on the finding that EPCs circulating in peripheral blood express the hematopoietic marker CD34.[32] Subsequent analysis confirmed that CD34$^+$ cells isolated from the bone marrow also differentiate into endothelial cells in vitro.[31] In vivo models of limb ischemia and myocardial infarction supported a direct role for bone marrow–derived cells in neovascularization, concluding that

these cells differentiate into smooth muscle and endothelial cells that incorporate into neovessels.[33–36] However, more recently, other groups have contested these findings and suggest that bone marrow–derived progenitor cells act primarily via paracrine mechanisms, secreting chemokines such as angiopoietin-1 and vascular endothelial growth factor (VEGF) at sites of vascular injury to enhance local angiogenic function.[27,29,30] It remains to be determined whether these discrepancies are attributable to differences in study design, such as the choice of bone marrow cell populations selected and the ischemic models used. Based on the current evidence, however, it appears that EPCs may make both direct contributions to neovascularization as well as indirectly promote the angiogenic function of local endothelial cells via secretion of angiogenic factors.

Bone marrow cells have also been shown to give rise to cardiomyocytes both in vitro[37–40] and in vivo.[35,41–44] Notably, the bone marrow was identified as a potential source of CSCs before CSCs were found in the heart itself.[42] Delivery of these cells, either systemically or intramyocardially, appears to result in cardiomyocyte differentiation that provides protection against myocardial infarction.[35,41,42,44,45] Importantly, there has been much debate regarding the validity of these results, with a number of groups suggesting that the "differentiation" of transplanted cells is in fact caused by fusion of the donor cells with host cells, producing binucleated cells with the characteristics of fully differentiated cardiac cell types.[46,47] The study of Oh et al, for example, demonstrated that although the stem cell–like population they had isolated was capable of differentiation into cardiac-like cell types when transplanted back into infarcted hearts, the authors acknowledged that as much as 50% of the "differentiated" cells had in fact undergone cell fusion, as demonstrated by using transgenic mouse models.[23] This finding, however, does not rule out the possibility that these cells may have undergone differentiation before fusion to mature cells. Indeed, the transplantation studies of Kajstura et al showed that although bone marrow cell transplantation after coronary artery ligation promotes regeneration of the infarcted myocardium, the cardiomyocytes that develop are not fully mature and are often poorly coupled and/or oriented in relation to endogenous myocytes.[48] This finding strongly suggests that these cells did not develop through fusion with existing myocytes within the infarcted region. Thus, although cell fusion may be a significant part of the process of cell engraftment after delivery to the heart, there is equally compelling evidence that true differentiation of CSC-like cells also contributes to the regeneration of the myocardium following cardiac ischemia.

The identification of CSCs located in the heart and CPCs in the bone marrow suggests that there may be a physiological link between these 2 stem cell niches. However, the relationship between bone marrow–derived and resident CSCs has yet to be established. Work from our group and others has demonstrated that many of the signaling mechanisms involved in cardiovascular repair in the heart itself are recapitulated in the bone marrow niche to generate or mobilize CPCs. Factors that have been identified both in the heart and bone marrow that may be involved in such mechanisms include stromal-derived factor-1,[49–52] VEGF,[53,54] granulocyte colony–stimulating factor (G-CSF),[16,55] and tenascin-C.[56,57] The parallels between the signaling pathways in the bone marrow and heart suggest that communication between these 2 tissues may be important for maintaining a reservoir of CSCs and CPCs in the bone marrow and local cardiac stem cell niches. Although it is possible that CSCs present in specialized niches of the myocardium take up residence during embryonic development and remain intact and unchanged over many decades, it is more likely that these cells undergo turnover and replenishment as they are called on periodically to respond to minor cardiac damage. However, it remains to be determined whether locally resident CSCs actually derive from the reservoir of CSCs present in the bone marrow. A recent study has demonstrated that after transplantation of green fluorescent protein–positive bone marrow cells that take up residence in the host bone marrow, subsequent induction of myocardial infarction results in a dramatic increase in the number of c-kit$^+$ cells in the heart and the majority of these are green fluorescent protein–positive.[58] Although this argues that bone marrow–derived cells have the capacity to home to the heart, where they express c-kit, the study does not determine whether these bone marrow–derived cells typically reside in the normal heart or whether they are recruited only in response to injury.

Aging and Cardiac Progenitor Cells

Because CSCs and CPCs appear to have the capacity to generate new cardiac tissues, or at least to enhance the function of existing cardiac cells, it remains to be determined why these cells fail to inhibit the progression of CVD. One explanation is that they have a limited capacity for repair and regeneration and that this capacity is insufficient to combat the effects of chronic disease. In addition, as CVD primarily affects the aging population, it is likely that both the number and function of CSCs and CPCs is impaired with increasing age and its associated pathologies that are caused by environmental factors as well as senescent changes within the cells themselves.

Cell senescence may be defined as the inability of cells to undergo replication, primarily as a result of genome instability (Figure 1). Senescence may be seen as an innate response to cell damage that is necessary to prevent tumor formation; however, the effects of senescence may also lead to disruption of normal organ function and progression of age-associated diseases. The rate of senescence within a cell is determined both by internal factors as well as environmental influences. Telomerase, which is critical for stabilizing the ends of chromosomes at cell division, has been shown to decrease with age in the heart and vasculature.[60] Telomerase-deficient mice experience premature aging of many of their organs. Moreover, postnatal angiogenic mechanisms are inhibited and cardiomyocyte proliferation is decreased in these mice, suggesting that CPC/CSC function is impaired.[61,62]

The accumulative effects of oxidative damage also promote senescent cell changes. The production of superoxide and hydrogen peroxide from mitochondria increases with age, as does the generation of peroxynitrite from nitric oxide and superoxide within the cell.[63] Additionally, circulating and

mitochondrial levels of antioxidant molecules such as superoxide dismutase and glutathione decrease with age, leading to an overall increase in oxidative stress.[63] The activity of the antioxidant enzyme glutathione peroxidase type 1 (GPx-1) has been shown to be downregulated in areas of atherosclerosis,[64] and recently, Galasso et al have demonstrated that mice deficient in the GPx-1 gene exhibit impaired EPC mobilization in response to ischemic injury or VEGF treatment, as well as impaired angiogenic function when transplanted into wild-type mice.[65] The presence of reactive oxygen species in a cell results in DNA damage. If damage to the nuclear DNA occurs, cells will either undergo apoptosis or DNA damage may induce oncogenic proteins such as p53, ras, and myc that subsequently upregulate cyclin-dependent kinase inhibitors, including p21 and $p16^{INK4a}$. The induction of these proteins ultimately leads to cell cycle arrest.[66]

External influences that deleteriously affect cell integrity or function may also promote cell senescence. Cells in regions of the vasculature that are more prone to atherosclerosis, for example, display a higher occurrence of senescence compared with cells in other regions of the endothelium. This is likely to be attributable, in part, to the high levels of shear stress in these areas that cause cellular damage.[67]

Aging and Resident Cardiac Stem Cells

Research in the area of resident CSCs is still in its early stages. However, recognizing the potential importance of this cell population in cardiac regeneration, Anversa et al have already begun to study the effects of aging and senescent changes in this cell population. Histological examination of cardiac tissue from patients with signs of CVD has shown that c-kit^+ resident CSCs undergo apoptosis and express the cyclin-dependent kinase $p16^{INK4a}$.[68] In mice, CSC apoptosis was more prevalent in older animals, and CSC telomere length also decreased with age.[69] These studies suggest that resident CSCs are indeed subject to senescent changes with increasing age, apparently in much the same way as mature cardiovascular cells. Although this may seem paradoxical, because stem cells by nature are self-renewing, the age-associated changes in resident CSCs may reflect the microenvironmental changes in the aging heart that impinge on CSC function. Clearly, further studies are required to establish whether CSC senescence contributes to the impairment in cardiac function and cardiovascular repair mechanisms in the aging patient. However, given that a number of studies have suggested that resident CSCs make a significant contribution to cardiovascular homeostasis and repair,[22,23,69] the aging of these cells is likely to have a major impact on cardiovascular health in older individuals.

Aging and Endothelial Progenitor Cells

The number of circulating EPCs in patients with coronary artery disease has been shown to decline with increasing age.[8] Furthermore, following coronary artery bypass grafting, EPC mobilization is significantly impaired in older individuals compared with younger patients.[8] Besides changes in EPC levels, the function of EPCs from older individuals also appears to be disrupted, based on in vitro examination of EPC survival, proliferation, and migration.[9,70] These results strongly suggest that age is an important determinant of EPC function and further support the hypothesis that changes in EPC function with age contribute to the impairment of cardiovascular repair mechanisms in the aging host.

A wide range of environmental changes influence EPC generation and function. The dramatic decrease in estrogen levels at the onset of menopause, for example, is associated with decreased levels of circulating EPCs and a drastic increase in CVD in postmenopausal women.[1,71] Mechanistically, estrogen increases telomerase activity and thus inhibits senescence in EPCs in vitro, which may partially explain the decrease in EPC levels at the time of menopause.[72] Furthermore, Strehlow et al have shown that there is both impaired generation and mobilization of EPCs from ovariectomized female mice and that estrogen replacement restores EPC levels in the bone marrow and peripheral blood via caspase 8-mediated antiapoptotic pathways.[73] A similar study showed that estrogen additionally promotes EPC migration and proliferative capacity.[74]

The age-associated impairment in EPC mobilization and function may related to changes in NO and reactive oxygen species. Specifically, endothelial NO synthase (eNOS) expression and subsequent nitric oxide production are critical in EPC mobilization.[75,76] Indeed, eNOS is a central downstream mediator in VEGF and estrogen-signaling pathways.[74,77,78] Moreover, oxidized low-density lipoprotein, which accumulates with age, also suppresses eNOS expression and impairs EPC survival and function.[79] Hypercholesteremia, a risk factor for CVD, is linked to decreased circulating EPC levels in humans as well as depressed EPC function in vitro, including impairments in cell migration, proliferation, and vasculogenic activity.[80] Smoking is similarly associated with decreased EPC levels and function,[70,81] whereas the cessation of smoking appears to induce rapid rises in EPC levels.[82] These studies highlight the fact that although CVD may have multifactorial etiologies, a common element is the decreased number and function of cardiovascular progenitor cells. Identifying factors that are downregulated with age (and its associated pathologies) and that are essential for maintenance of progenitor cell number and function may therefore provide novel targets for the restoration of cardiovascular repair mechanisms in older individuals.

Cardiovascular Disease and Cardiac Progenitor Cells

The impact of the impairment in EPC function has been studied in the context of various vascular pathologies, which shed light on the important, and often complex, role of these cells in vascular regeneration. Furthermore, the progressive loss of myocytes in the aging and infarcted heart, as myocyte damage and apoptosis begin to outpace cardiac remodeling, suggest that future therapies for the treatment of myocardial damage may involve the use of CPCs and CSCs to replace lost myocytes and their associated vasculature.

Atherosclerosis

Atherosclerosis results from endothelial cell damage, often as a result of inflammation or physical disruption to the vascular surface, which leads to loss of integrity of the endothelial

monolayer. This damage is followed by infiltration of inflammatory cells that can promote endothelial cell apoptosis and dysfunction, as well as lipid deposition and smooth muscle cell proliferation that result in neointima formation.[83] Regeneration of the endothelium is therefore an obvious target for preventing the development of atherosclerosis. A number of studies have demonstrated that transplantation of EPCs, or mobilization of endogenous EPCs using agents such as statins and G-CSF, contribute to the reendothelialization of denuded vessels in vivo, reducing neointima formation.[84–87] The study of Rauscher et al further highlights the importance of aging in the EPC-mediated prevention of atherosclerosis: this group demonstrated that the treatment of old ApoE$^{-/-}$ mice with bone marrow cells from young ApoE$^{-/-}$ mice was able to inhibit the progression of atherosclerosis, whereas cells from older ApoE$^{-/-}$ mice, which themselves had signs of atherosclerosis, did not.[88]

Although EPCs may be important for replacement of damaged cells at the vascular surface, subsequent maintenance and growth of the atherosclerotic lesion involves vascularization of the neointimal tissue, a process that is likely to be enhanced by the presence of EPCs. Indeed, George et al have shown that transplantation of bone marrow cells or spleen-derived EPCs into ApoE$^{-/-}$ mice promotes atherosclerotic lesion development.[89] Vessel density has also been shown to be highest in atherosclerotic lesions that show evidence of hemorrhage, plaque rupture and severe inflammation, suggesting that neovascularization is linked to plaque vulnerability.[90] Furthermore, it has been reported that bone marrow cell transplantation following in vivo endothelial denudation or cardiac transplantation leads to differentiation of smooth muscle cells that contribute to the development of atherosclerotic lesions and exacerbate vascular pathology.[91] These studies point to a need for the development of therapies that can target regions of vascular dysfunction in a spatially and temporally controlled manner, maintaining a balance between promotion of tissue regeneration and inhibition of aberrant growth.

Myocardial Infarction

Acute myocardial infarction in humans is associated with increased levels of circulating EPCs.[33] This upregulation, however, generally appears to be insufficient to prevent cardiovascular damage. This is likely attributable, in part, to the delayed response, such that EPC mobilization does not peak until 7 days after vascular injury.[33] Mobilization of cardiac progenitors before or at the time of myocardial infarction may promote increased myocyte differentiation and/or tissue vascularization. We have shown that pretreatment of mice with a combination of angiogenic growth factors, platelet-derived growth factor (PDGF) and VEGF, 24 hours before cardiac allograft transplantation promotes both local angiogenic and EPC-mediated vasculogenic responses that are impaired in aging mice, resulting in successful allograft vascularization.[92] This is in contrast to growth factor administration at the time of transplantation, which is significantly less successful.[92] Similarly, myocardial pretreatment with PDGF before coronary artery ligation promotes cardiomyocyte differentiation from bone marrow cells that limits the extent of myocardial infarction.[44] Thus, early or preventative interventions may prove to be the most efficient strategies in individuals with susceptibility to CVD.

Urbanek et al have attempted to enhance the homing of endogenous CSCs by injecting hepatocyte growth factor (HGF) and insulin-like growth factor (IGF)-1 to attract c-met–positive and IGF-1 receptor–positive CSCs to sites of myocardial infarction.[93] This resulted in regeneration of functionally competent myocytes and associated vascular structures within the infarct region and subsequent improvement in cardiac performance.[93] Together, these studies demonstrate that the host has the capacity to regenerate its own cardiac and vascular tissues after injury but may be inhibited from doing so because of impairment of the necessary signaling mechanisms. In aging hosts, this may be compounded by the fact that the pool of CPCs and CSCs is probably depleted, thus transplantation of bone marrow cells or specific progenitor cell populations to restore the numbers of these cells also promotes cardiovascular repair. Both experimental studies and clinical trials examining the effectiveness of cell replacement approaches have provided promising results that suggest that progenitor cell transplantation may be a viable strategy for the treatment of CVD (see below).

Diabetes

Diabetes is linked to impaired vascular function, including alterations in both endothelial cells and EPCs. A number of studies have shown that individuals with diabetes have decreased levels of circulating EPCs[94–96] and that the severity of disease is inversely proportional to EPC levels.[97] In vitro, hyperglycemia increases the rate of EPC senescence[98] and the angiogenic function of EPCs from patients with either type 1 or type 2 diabetes is impaired such that they are poorly proliferative and fail to incorporate into forming vessel-like structures.[97,99] Our group has shown that whereas bone marrow transplantation from young wild-type mice into old wild-type mice is able to restore the ability of the aging host to vascularize a cardiac allograft, similar studies using bone marrow cells from young diabetic mice fail to restore angiogenic potential.[100] Treatment of the diabetic donor cells with PDGF was able to rescue the angiogenic capacity of the cells, demonstrating that not only is a lack of cell mobilization from the bone marrow a cause of EPC dysfunction in diabetic animals but dysregulation of growth factor-mediated signaling is partly responsible for the loss of angiogenic function. Similarly, bone marrow cells from diabetic mice display decreased levels of VEGF expression and increased thrombospondin-1 expression, which are likely linked to the impaired angiogenic capacity of these cells.[101] Thus, as with myocardial infarction, the use of growth factor and cytokine-mediated approaches may prove successful in the development of strategies to improve vascular function in diabetic patients.

Progenitor Cell–Mediated Repair of the Aging Cardiovascular System

The extent to which CSCs and CPCs appear to be involved in the maintenance of endogenous cardiovascular repair mech-

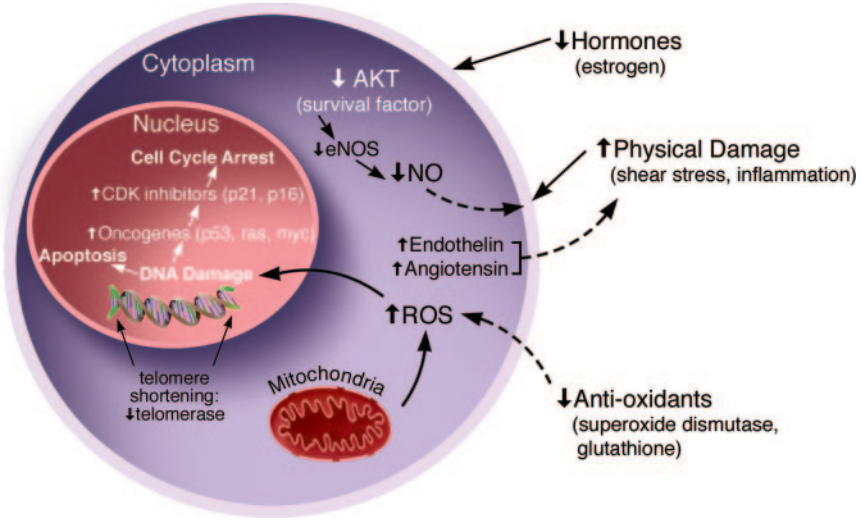
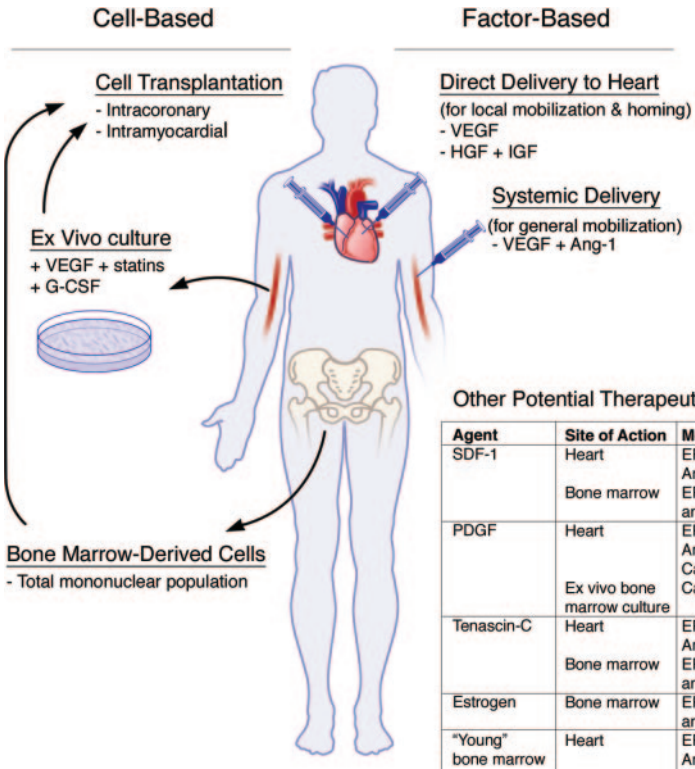


Figure 1. Influences on cell senescence. Senescent changes in cells result in the cessation of cell replication, often in response to cell damage and/or genome instability. The changes illustrated here are thought to promote senescence and have been suggested to contribute to the progressive senescence of CPCs and, in some cases, CSCs. Reversal or inhibition of these senescent pathways may provide feasible strategies to maintain the pool of CPCs and CSCs in the aging host, thus maintaining regenerative and cardioprotective pathways. eNOS indicates endothelial nitric oxide synthase; ROS, reactive oxygen species.

anisms highlights the potential importance of these cell types in the development of new therapies to reduce or prevent the impact of CVD in the aging population. A number of different CSC-/CPC-based approaches are showing promising results in the laboratory and, in some cases, in clinical trials. These approaches can be broadly separated into 2 categories: those that use cell transplantation methods to replenish CSC/CPC pools and replace damaged tissues and those that use factor-based approaches to restore molecular pathways that are dysregulated with age and enhance the regenerative function of endogenous cells (Figure 2).

Cell-Based Approaches for Cardiovascular Repair

Approaches to cardiac cell replacement include direct injection of cells at sites of vascular injury as well as delivery of cells to the systemic circulation, relying on the fact that homing of progenitor cells to injured cardiac and vascular tissues is significantly higher than engraftment to uninjured sites.[102] Our laboratory has focused on the restoration of the aging bone marrow stem cell niche, ie, the microenvironment in which bone marrow progenitor cells are generated and mobilized, as a means to revert the aging bone marrow to a "young" phenotype with CPC-mediated vasculoprotective



Other Potential Therapeutic Agents

Agent	Site of Action	Mode of Action
SDF-1	Heart	EPC homing Angiogenic induction
	Bone marrow	EPC generation and angiogenic function
PDGF	Heart	EPC homing Angiogenic induction Cardiomyogenesis
	Ex vivo bone marrow culture	Cardiomyogenesis
Tenascin-C	Heart	EPC homing Angiogenic induction
	Bone marrow	EPC generation and angiogenic function
Estrogen	Bone marrow	EPC generation, survival and function
"Young" bone marrow	Heart	EPC generation Angiogenic induction Cardiomyogenesis
	Bone marrow	EPC generation
	Ex vivo bone marrow culture	EPC generation Cardiomyogenesis

Figure 2. Potential clinical approaches to regeneration of the aging cardiovascular system. Current clinical trials aimed at the enhancement of cardiac regeneration in patients with cardiovascular disease are focused on 2 major strategies: (1) the direct delivery of CPCs to the heart itself, either with or without prior ex vivo culture to enhance cardiac differentiation; and (2) the enhancement of endogenous CPC and/or CSC function via the delivery of factors that promote both the mobilization and homing of these cells to the site of injury. There are also a number of agents that show promise in experimental studies for the promotion of CPC and CSC function, including SDF-1, tenascin-C, and estrogen. Additionally, the use of exogenous sources of stem/progenitor cells with a "young" phenotype may also prove useful for restoration of cardiac regenerative properties that are progressively dysregulated with age.

capacity. We have shown that whereas old (18-month) mice lack the capacity to vascularize cardiac allografts, intravenous delivery of bone marrow cells from young (3-month) mice to old mice one week before cardiac transplantation results in increased cardiac angiogenic capacity, sufficient to support allograft vascularization.[103] Thus, the supplementation of old EPCs with young EPCs, without the need for myeloablation, is an effective strategy for restoring mechanisms of angiogenesis and cardiovascular repair in the aging heart.

Rather than restoring the bone marrow cell reservoir of CPCs/CSCs, other groups have examined the effectiveness of direct delivery of cells to the infarcted heart. Such approaches may lend themselves more favorably to clinical applications, because issues of cell rejection can be avoided through use of autologous cell transplantation. Strauer et al performed the first small-scale clinical study involving twenty patients with acute myocardial infarction, transplanting autologous bone marrow mononuclear cells into the infarcts of half the patients approximately 7 days after onset of infarction, coupled with standard therapies for all patients.[20] After 3 months, those patients who had undergone cell transplantation as well as standard therapy had significantly smaller infarcts with improved cardiac function, compared with the control group. Significantly, no apparent deleterious effects were observed in patients who underwent cell transplantation.

The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trial involved a similar approach to that used by Strauer et al,[20] except that 2 different cell populations were compared: (1) blood-derived mononuclear cells supplemented with both VEGF and atorvastatin and cultured ex vivo for 3 days; and (2) bone marrow–derived mononuclear cells, positive for both EPC and hematopoietic progenitor cell markers, transplanted without culturing.[12] Four months after treatment, again, those patients who had undergone cell transplantation displayed improved cardiac function with no negative effects. Interestingly, no differences in cardiac improvement were seen between patients receiving blood-derived versus bone marrow-derived mononuclear cells. A follow-up study by this group examined changes in infarct size with cell-based therapy and found that this was significantly decreased compared with infarct size in patients receiving only standard therapy.[13]

Since these clinical trials, other trials have shown similar positive outcomes after bone marrow transplantation in patients with myocardial ischemia.[104–106] Furthermore, a number of groups have examined the utility of pretreatment of patients with drugs such as G-CSF to stimulate bone marrow cell mobilization, followed by harvesting from the peripheral blood and injection into the ischemic heart.[14,17,18] Although these were very small-scale pilot studies, this approach appears to be safe and provides some cardiovascular benefit, suggesting that combined approaches using cytokine administration followed by CSC/CPC delivery may also prove to be a valuable therapeutic strategy to reduce the impact of ischemic heart disease.

The clinical trials described above illustrate the potential benefits of CSC/CPC transplantation to patients with CVD. Although there is great enthusiasm for these approaches, these promising results should be viewed with caution, because the long-term effects of bone marrow– and blood-derived cell transplantation are completely unknown. Potential problems that may arise include aberrant differentiation of the transplanted cells either (1) into unexpected cell types or (2) in unexpected patterns and locations. Many of the cell preparations that are used for these studies are incompletely characterized; thus differentiation into other cell types is feasible, particularly under pathological conditions. A more thorough analysis of the cell types involved, as well as their plasticity, is essential to ensuring that this is avoided. Related to this, the majority of transplanted cells will not home to the site of myocardial damage, but will instead home to other tissues, most notably the spleen and liver or, alternatively, will undergo apoptosis. The fate of cells that become engrafted into other tissues is unknown but may lead to disruption in organ function. Furthermore, aberrant differentiation of EPCs may promote tumor angiogenesis in susceptible patients, as seen in experimental studies.[107] Thus, a balance must be maintained between angiogenic induction to promote cardiovascular health and angiogenic suppression that may stimulate tumor growth.

Enhancement of Endogenous Function of CSCs and CPCs via Cytokine/Growth Factor Stimulation

Similar to the role of factor-based therapies to promote endogenous hematopoietic stem cell function, the future of cardiac regeneration and cardioprotection may lie in cell-free treatments to enhance the function of endogenous CSCs and CPCs. A number of factors have been shown to enhance EPC function either at the site of mobilization from the bone marrow and/or at sites of homing to damaged blood vessels. Age-associated decreases in a wide range of factors, including VEGF and PDGF signaling and circulating estrogen levels have been suggested in animal models to be important factors in the decrease in EPC mobilization from the bone marrow, cardiac homing, and regeneration.[71,103,108] Restoration of these factors in the aging host may provide protective benefit to the cardiovascular system that limits the impact of CVD.

VEGF is a key factor in angiogenic and vasculogenic mechanisms in both development and disease.[109,110] The systemic administration of VEGF in animal models and clinical trials results in increased mobilization of EPCs from the bone marrow and EPC proliferative and migratory activity and promotes incorporation of EPCs into sites of neovascularization in vivo.[32,111–115] Furthermore, a number of studies have shown that although intramyocardial transplantation of bone marrow cells alone promotes cardiac neovascularization and improves cardiac function after myocardial infarction, the results are even further enhanced if the bone marrow cells are transfected with VEGF-encoding plasmids before transplantation.[15,21,116–118] Clinical trials in patients with coronary artery disease or limb ischemia showed improvement after treatment with plasmid DNA encoding $VEGF_{165}$[15,21,117,118]; thus VEGF may prove to be a feasible and successful therapy for vascular injury. Importantly, studies such as these highlight the fact that replenishment of

cardiomyocytes by CSC/CPC transplantation after infarction may not be necessary if the infarcted tissue is revascularized promptly. EPCs enhance the survival of existing myocytes and may also induce recruitment of endogenous CSCs, as has been shown experimentally.[28,119] Thus, therapeutic approaches that focus on enhancement of EPC activity or restoration of the EPC population may be sufficient to improve cardiac structure and function after vascular injury.

Our laboratory has shown that PDGF acts to promote the angiogenic activity of local vascular cells after myocardial infarction as well as to recruit bone marrow cells that differentiate into both endothelial cells and cardiomyocytes.[44,120] Intramyocardial treatment with PDGF therefore appears to enhance the interactions between bone marrow and cardiac stem cell niches and provides functional benefit to the injured heart. Additionally, we have recently demonstrated that PDGF pathways are essential for maintaining the cardiomyogenic potential of Oct3/4$^+$ bone marrow cells that is decreased with age.[121] Although the direct use of PDGF as a therapeutic strategy is not feasible because of its known ability to induce smooth muscle cell differentiation,[122] similar agents that promote parallel pathways in the bone marrow and heart may prove to be the most beneficial for enhancing progenitor cell function. Tenascin-C, which we have shown to be a downstream mediator of PDGF signaling in the cardiac vasculature, is associated with sites of EPC recruitment in the heart and is also important for bone marrow cell–mediated mechanisms of cardiac angiogenesis.[56] This protein is downregulated in the aging bone marrow and may also be depleted in the aging heart.[56] Thus mechanisms that restore tenascin-C may have multiple actions that promote cardiovascular repair mechanisms, including CSC-mediated cardiac regeneration.

Another factor acting both in the cardiac and bone marrow stem cell niches is stromal cell–derived factor (SDF)-1. In the bone marrow, SDF-1 is among a number of proteins, including VEGF and placental growth factor, that induce matrix metalloproteinase (MMP)-9, leading to the translocation of stem cells to the vascular zone of the bone marrow before mobilization.[123] SDF-1 has also been shown to promote bone marrow cell proliferation and angiogenesis.[124] In vitro, EPCs migrate toward SDF-1 and injection of SDF-1 into sites of limb ischemia, combined with EPC transplantation, promotes local EPC-mediated vasculogenesis.[125] Furthermore, this factor can also suppress EPC apoptosis.[125] In the heart, SDF-1 is upregulated immediately after myocardial infarction, suggesting that, here, SDF-1 may act as a homing signal to recruit cardiac progenitor cells for tissue repair.[51] Like SDF-1, the growth factor IGF-1 is also known to have multiple effects on CSCs and CPCs that make it a potential therapeutic target for the maintenance of cardiac regeneration in the aging host. In the heart, the functions of IGF-1 act to preserve the integrity of existing cardiomyocytes, inhibiting replicative senescence and apoptosis, in part via upregulation of telomerase activity and antioxidant pathways.[68,126,127] Moreover, Torella et al have demonstrated that IGF-1 overexpression also acts to maintain the pool of CSCs in the heart, by inhibition of apoptosis and suppression of cell cycle inhibitors.[69] As mentioned above, IGF-1 has been used in experimental studies to enhance the homing of endogenous IGF-1 receptor–positive CSCs.[93] This factor may therefore prove useful not only for recruitment of CSCs to sites of cardiac injury but also for maintaining their viability and function on incorporation into the healing myocardium.

Other factors that regulate EPC function include the hematopoietic growth factor granulocyte macrophage–colony stimulating factor, which increases the number of circulating EPCs in vivo, while enhancing differentiation of EPCs in vitro.[128] Similarly G-CSF also has stimulated EPC mobilization in clinical trials, but these EPCs display functional impairment of migratory properties in vitro. As a result, there is no apparent improvement in individuals treated with G-CSF after myocardial infarction.[16,19] These studies suggest that cytokine administration may need to be combined with CSC/CPC delivery to develop an improved therapeutic strategy to reduce the impact of ischemic heart disease specifically in older persons.

Pharmacologically, the class of factors known as the statins, or 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitors, has also been shown to enhance EPC-mediated angiogenesis in models of ischemic tissue injury. In vivo, statin treatment increases the numbers of circulating EPCs and enhances both neovascularization in corneal assays and reendothelialization of injured vessels, promoting incorporation of labeled bone marrow–derived cells into these vessels.[84,86,129] Mechanistically, statin treatment in vitro appears to inhibit EPC senescence, via induction of telomere repeat binding factor-2, which inhibits induction of the DNA damage checkpoint-kinase 2.[130] Simvastatin activates the serine–threonine kinase Akt in endothelial cells, promoting endothelial cell survival and migration.[131] Akt also acts downstream of VEGF and may therefore represent a key regulator of VEGF-mediated neovasculogenesis.[132] Thus, these data suggest that statin therapy may constitute an important approach in the development of strategies to improve EPC survival and function and to improve cardiac repair pathways in the aging population. Indeed, the TOPCARE-AMI clinical trial demonstrated that the treatment of ex vivo cultured blood-derived progenitor cells with atorvastatin was found to be safe and potentially effective for the enhancement of cardiac regeneration.[12]

Concluding Remarks

Since the discovery that the heart is capable of regeneration, much of the research in this field has focused on the characterization of these stem and progenitor cells and the signaling mechanisms that promote their generation and function. Early clinical trials suggest that the application of CPCs and/or CSCs for cardiovascular repair may be a safe and viable alternative to current strategies. However, the modest functional improvements observed in these trials may be just the starting point for realizing the full potential of these cells to repair cardiovascular damage. Until now, the majority of experimental studies have focused on investigation of CSC and CPC function in young hosts. To use these cells most effectively for cardiovascular repair, the next phase of research in this field must be aimed at understanding the deterioration of endogenous CSC and CPC function in the

context of aging that inhibits their effectiveness at repairing cardiovascular damage. Research into the senescent changes in these cell populations, as well as the environments in which they are generated and to which they are recruited, will yield valuable insight into the mechanisms that are essential to the maintenance of their regenerative function. Moreover, identification of signaling pathways that are dysregulated with age will provide molecular targets for the development of treatments to enhance the potential of endogenous CSCs and CPCs and/or to enhance the functional capacity of transplanted cells. The promising studies described within this review suggest that future therapeutics based on our increased understanding of the biology of stem and progenitor cells in the context of aging will lead to improved outcomes for older individuals with CVD and will likely be beneficial for the development of therapies for all individuals with heart disease.

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Disclosures

None.

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