The Indonesian Biomedical Journal, Vol.1, No.2, August 2009, p.4-23 Print ISSN: 2085-3297, Online ISSN: 2355-9179

REVIEW ARTICLE

Endothelial Progenitor Cells in Diabetic Vasculopathy

Anna Meiliana[1,2]* and Andi Wijaya[1,2]*

Abstract

BACKGROUND: The discovery of endothelial progenitor cell (EPC) a decade ago by Asahara et al has refuted the previous belief that vasculogenesis only occurs during embryogenesis. The reduced circulating concentration of EPCs is a surrogate marker of endothelial function and has been implicated in the pathogenesis of many vascular diseases.

CONTENT: 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 level of circulating EPCs and that the severity of disease is inversely proportional to EPC levels. In vitro, hyperglycemia increases the rate of EPC senescence 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.
Given the comprehensive role of EPC alterations in diabetes complications, modulation of the levels and/or function of EPCs may be considered a potential therapeutic strategy.

SUMMARY: The available data demonstrating that decrease or dysfunction of EPCs may have a prominent role in the pathogenesis of all diabetes complications. Further approaches, such as EPC administration, may represent novel treatments for diabetic vasculopathy in the future. To date, many barriers remain to such a therapeutic approach. Firstly, there is no specific marker for EPC at present. Secondly, techniques of EPC isolation are not standardized, preventing direct comparison between various studies. The long-term effects of transplanted EPCs are currently unknown.

Introduction

EPCs can promote postnatal vasculogenesis by homing to, differentiating, proliferating and incorporating into new vessels. EPCs may play a role in this process by physically incorporating or may influence neighbouring cells in a paracrine fashion. These normal roles of EPCs may explain why these cells have been implicated in the pathogenesis of many vascular disease states such as ischaemia, restenosis and pulmonary hypertension (1-6). EPC number and function can be affected by various cardiovascular risk factors such as cigarette smoking, hypertension and hyperlipidaemia (7–9). In fact, Hill et al. have demonstrated that EPC number may be a surrogate biologic marker for vascular function and cumulative cardiovascular risk in healthy men (10). Schmidt-Lucke et al. and Werner et al. later showed that the number of circulating EPCs can predict the occurrence of cardiovascular events and death from cardiovascular causes and may help to identify patients at increased cardiovascular risk (11,12). EPC dysfunction has also been observed in the high cardiovascular risk state of diabetes mellitus (DM).

[1]Post Graduate Program in Clinical Biochemistry, Hasanuddin University, Makassar
[2]Prodia Clinical Laboratory, Jakarta

*Address correspondence to this author at: Prodia Clinical Laboratory, Jl. Cisangkuy No. 2 Bandung; e-mail: anna_m@prodia.co.id, andi_w@prodia.co.id

The impaired neovascularization found in the diabetic setting may be explained by the inability of diabetic EPCs to respond to stromal-derived factor-1 (SDF-1), which plays a crucial role in EPC homing (13). Dysfunctional EPCs in the diabetic setting are also associated with impaired re-endothelialization (14). Furthermore, EPC mobilization was also impaired after ischaemic reperfusion injury in a streptozotocin induced diabetic mouse model, a finding that was associated with altered SDF-1 and VEGF release as well as impaired hypoxia inducible factor-1a upregulation (15). These in vivo animal studies demonstrated that diabetes is associated with impaired EPC mobilization, differentiation and ability to form tubules.

The administration of both autologous and heterologous unfractionated BM cells, which contain EPCs, has produced dramatic improvement in neovascularization in various ischaemic states (16-26). However, the results of autologous EPC transplantation in the diabetic setting may not be as promising. As discussed above, in patients with DM, EPC numbers are lower and their function is impaired.

Diabetic Complications

Diabetes complications represent a huge burden for patients and health services. The fight against each single complication has led to significant improvements in diabetes care, especially for microvascular complications, yet macroangiopathy remains a major source of morbidity and mortality. A common approach for the prevention and treatment of diabetes complications relies on the understanding of their complex pathophysiology (27).

While it was originally thought that a single patient tends to develop the cluster of micro- or macrovascular complications, recent prospective studies show that typical markers of microvascular dysfunction, such as microalbuminuria or retinal vascular abnormalities, are associated with an increased risk of macrovascular events (28,29). These and other data suggest that there must be a unifying pathogenetic model underlying diabetes complications. To date, the most credited and supported model proposes that oxidative stress originating from mitochondria activates all subcellular damage pathways (30). However, subsequent events diverge for each complication, and there is not a supracellular unifying hypothesis. The discovery that a subset of circulating immature cells contributes to vascular homeostasis has been a major achievement in many fields of basic science.

Endothelial progenitor cells (EPCs) are circulating immature cells that contribute to vascular homeostasis and compensatory angiogenesis. During the last decade, data have become available indicating that alterations in EPCs may have an important causative role in the development and progression of virtually all diabetes complications (27).

Endothelial Progenitor Cells (EPCs)

EPCs were discovered in 1997 as circulating cells with the ability to differentiate into mature endothelium and take part in neoangiogenesis (1). EPCs share markers of hematopoietic (CD34 and CD133) and endothelial (KDR, CD31, and vWf) lineages (31), are derived from bone marrow, and can be mobilized to the peripheral circulation in response to many stimuli (32). Tissue ischemia, through the release of growth factors and cytokines, mobilizes EPCs, which, once in the peripheral circulation, specifically home on the ischemic sites to stimulate compensatory angiogenesis (33). Moreover, EPCs constitute a circulating pool of cells able to form a cellular patch at sites of endothelial injury, thus contributing directly to the homeostasis and repair of the endothelial layer. Taken together, these observations suggest that EPCs have a major role in cardiovascular biology; in fact, the extent of the circulating EPC pool is now considered a mirror of cardiovascular health. Virtually all risk factors for atherosclerosis have been associated with decrease and/or dysfunction of circulating EPCs (34), while an expanded EPC pool is associated with a decreased cardiovascular mortality (11).

According to the initial discovery, EPCs or CEPCs (Circulating bone marrow – derived endothelial progenitor cells) were defined as cells positive for both hematopoietic stem cell markers such as CD34 and an endothelial marker protein as VEGFR2. Because CD34 is not exclusively expressed on hematopoietic stem cells but, albeit at a lower level, also on mature endothelial cells, further studies used the more immature hematopoietic stem cell marker CD133+ and demonstrated that purified CD133+ cells can differentiate to endothelial cells in vitro (35). CD133, also known as prominin or AC133, is a highly conserved antigen with unknown biological activity, which is expressed on hematopoietic stem cells but is

absent on mature endothelial cells and monocytic cells (36) Thus, CD133+VEGFR2+ cells more likely reflect immature progenitor cells, whereas CD34+VEGFR2+ may also represent shedded cells of the vessel wall. At present, it is unclear whether CD133 only represents a surface marker or has a functional activity involved in regulation of neovascularization. Overall, controversy exists with respect to the identification and the origin of endothelial progenitor cells, which are isolated from peripheral blood mononuclear cells by cultivation in medium favoring endothelial differentiation. In peripheral blood mononuclear cells, several possible sources for endothelial cells may exist: (1) the rare number of hematopoietic stem cells, (2) myeloid cells, which may differentiate to endothelial cells under the cultivation selection pressure, (3) other circulating progenitor cells (eg, "side population" cells), and (4) circulating mature endothelial cells, which are shed off the vessel wall (37) and adhere to the culture dishes (38).

A combination of CD34, kinase insert domain-containing receptor (KDR) and CD133 are commonly used to define EPCs at present. The use of fluorescence-activated cell sorter (FACS) analysis to define EPCs appears to be the most optimal approach but may be technically challenging and the best combination of the 'true' markers for EPCs is currently unknown (39).

The study of EPC biology consists of two related aspects: quantitative evaluation of the EPC pool and functional assessment. Circulating EPCs can be quantified directly ex vivo using flow cytometry, which is considered the gold standard for this purpose (40); typical surface antigens to identify EPCs are CD34, CD133, and KDR. Functional characteristics are explored in vitro using standardized protocols (38). Proliferation refers to the ability of EPCs to expand and form colonies in culture: EPCs should proliferate in response to growth factors released locally after vascular damage or tissue ischemia. Adhesion is a further step required for both reendothelization and angiogenesis; it is assessed as the ability of EPCs to adhere to a monolayer of mature endothelium in culture. Migration of EPCs through the extracellular matrix is crucial for the growth of new vessels and is generally assessed in vitro as the ability to invade the lower side of a Boyden-like chamber. Finally, after EPCs have adhered to the vessel wall, migrated into the interstitium, and expanded locally, they should spatially organize to form vascular structures; this property can be assessed in vitro as a tube formation assay in which EPCs are seeded with human umbilical vein endothelial cells on a gel of extracellular matrix proteins.

Two types of EPC had different morphology, proliferation rates, and survival behaviors. They also had different gene expression profiles, leading to different function in vitro. Despite such differences in gene expression and in vitro function, they equally contributed to neovasculogenesis in vivo in that early EPC secreted angiogenic cytokines, whereas late EPC supplied a sufficient number of endothelial cells (41).

Late EPC was different from early EPC in the expression of VE-cadherin, Flt-1, KDR, and CD45. Late EPC produced more nitric oxide, incorporated more readily into human umbilical vein endothelial cells monolayer, and formed capillary tube better than early EPC. Early EPC secreted angiogenic cytokines (vascular endothelial growth factor, interleukin 8) more so than late EPC during culture in vitro. Both types of EPC showed comparable in vivo vasculogenic capacity (41).

Distinct origins of the different types of EPCs exist that have different functions in neovascularization. Mixed transplantation of these cells results in synergistic neovascularization through cytokines and MMPs (42).

EPCs in Neovascularization

Improvement of neovascularization is a therapeutic option to rescue tissue from critical ischemia (43). The finding that bone marrow–derived cells can home to sites of ischemia and express endothelial marker proteins has challenged the use of isolated hematopoietic stem cells or EPCs for therapeutic vasculogenesis.

However, the number of incorporated cells with an endothelial phenotype into ischemic tissues is generally quite low. How can such a small number of cells increase neovascularization? A possible explanation might be that the efficiency of neovascularization may not solely be attributable to the incorporation of EPCs in newly formed vessels, but may also be influenced by the release of proangiogenic factors in a paracrine manner (38).

Thus, EPCs may act similar to monocytes/macrophages, which can increase arteriogenesis by providing cytokines and growth factors. Indeed, EPCs cultivated from different sources showed a marked

expression of growth factors such as VEGF, HGF, and IGF-1. Moreover, adherent monocytic cells, which were cultivated under similar conditions, but do not express endothelial marker proteins, also release VEGF, HGF, and G-CSF (44). The release of growth factors in turn may influence the classical process of angiogenesis, namely the proliferation and migration as well as survival of mature endothelial cells (45). However, EPCs additionally incorporated into the newly formed vessel structures and showed endothelial marker protein expression in vivo.

EPCs in Endothelial Regeneration

In the past, the regeneration of injured endothelium has been attributed to the migration and proliferation of neighboring endothelial cells. More recent studies, however, indicate that additional repair mechanisms may exist to replace denuded or injured arteries.

Bone marrow transplantation experiments revealed that bone marrow–derived cells can contribute to reendothelialization of grafts and denuded arteries (46-48). However, in a model of transplant arteriosclerosis, bone marrow–derived cells appear to contribute only to a minor extent to endothelial regeneration by circulating cells (49). These data again indicate that there might be at least two distinct populations of circulating cells that principally are capable to contribute to reendothelialization, namely mobilized cells from bone marrow and non-bone marrow–derived cells. The latter ones may arise from circulating progenitor cells released by non-bone marrow sources (eg, tissue resident stem cells) or represent vessel wall–derived endothelial cells (49, 46-48).

A rapid regeneration of the endothelial monolayer may prevent restenosis development by endothelial synthesis of antiproliferative mediators such as nitric oxide. Indeed, enhanced incorporation of β-galactosidase–positive, bone marrow–derived cells was associated with an accelerated reendothelialization and reduction of restenosis (46,47). Similar results were reported by Griese et al, who demonstrated that infused peripheral blood monocyte–derived EPC home to bioprosthetic grafts and to balloon-injured carotid arteries, the latter being associated with a significant reduction in neointima deposition (50). Likewise, infusion of bone marrow–derived CD34-/CD14+ mononuclear cells, which are not representing the population of the "classical hemangioblast," contributed to endothelial regeneration (51). The regenerated endothelium was functionally active as shown by the release of NO (51), which is supposed to be one of the major vasculoprotective mechanisms. Consistently, neointima development was significantly reduced after cell infusion (51). Whereas the regeneration of the endothelium by EPCs protects lesion formation, bone marrow–derived stem/progenitor cells may also contribute to plaque

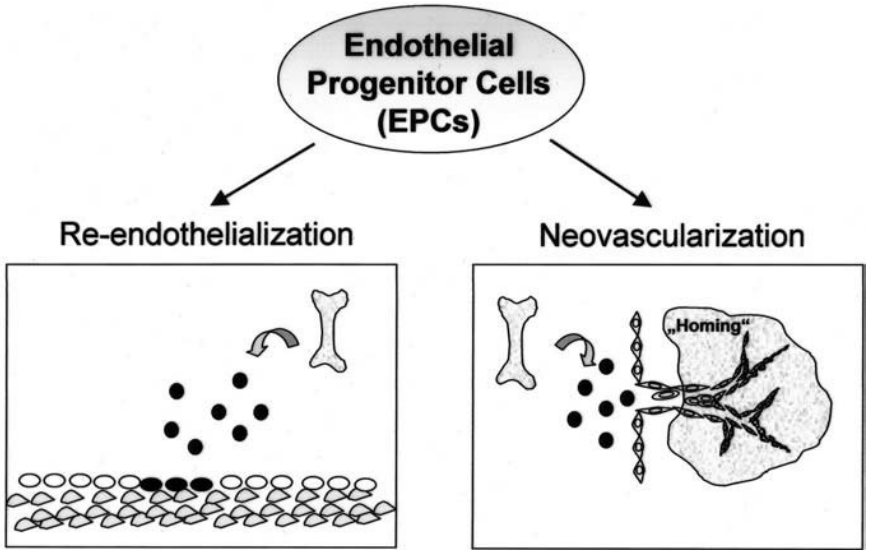

Figure 1. Role of EPCs in vascular biology. Reprinted by permission from Wolter Kluwer Health, Circ.Res, Copyright (2004).

angiogenesis, thereby potentially facilitating plaque instability (52). However, in a recent study, no influence of bone marrow cell infusion on plaque composition was detected in nonischemic mice (53). An increase in plaque size was only detected in the presence of ischemia, suggesting that ischemia-induced release of growth factors predominantly accounts for this effect (53).

Overall, these studies implicate that regardless of the origin of circulating endothelial progenitor cells, this pool of circulating endothelial cells may exert an important function as an endogenous repair mechanism to maintain the integrity of the endothelial monolayer by replacing denuded parts of the artery (Figure 1). One can speculate that these cells may also regenerate the low grade endothelial damage by ongoing induction of endothelial cell apoptosis induced by risk factors for coronary artery disease (54).

Moreover, various risk factors for coronary artery disease, such as diabetes, hypercholesterolemia, hypertension, and smoking, affect the number and functional activity of EPCs in healthy volunteers (10) and in patients with coronary artery disease (55).

In addition, factors that reduce cardiovascular risk such as statins (56,46,47,57) or exercise (58) elevate EPC levels, which contribute to enhanced endothelial repair. The balance of atheroprotective and proatherosclerotic factors, thus, may influence EPC levels and subsequently reendothelialization capacity.

EPCs Mobilization Differentiation and Homing

The release of EPCs from the bone marrow is regulated by a variety of growth factors, enzymes, ligands, and surface receptors. Activation of matrix metalloproteinase-9, which promotes the transformation of membrane-bound Kit ligand to a soluble Kit ligand and the subsequent move of cKit-positive stem and progenitor cells, including a common hematopoietic and angioblast precursor (hemangioblast), to the vascular zone of the bone marrow microenvironment, are initial steps in this complex process (59). The signals, which initiate the diversion of the hemangioblast to either hematopoietic precursor cells or EPCs, are largely unknown at present but may include angiogenic growth factors from the periphery.

To date, no clear definition exists as to when an endothelial progenitor cell turns into a mature, fully differentiated endothelial cell in vivo. One possibility could be the loss of CD133 and a parallel or subsequent expression of von Willebrand factor in conjunction with the appearance of other endothelial characteristics. The starting point of this differentiation process may by the migration of EPCs from the bone marrow to the systemic circulation. After homing, ie, after adhesion and insertion into the monolayer of surrounding mature vascular ECs, this process may be completed (60).

Recruitment and incorporation of EPCs requires a coordinated sequence of multistep adhesive and signaling events including chemoattraction, adhesion, and transmigration, and finally the differentiation to endothelial cells (Figure 2) (38).

The local bone marrow microenvironment, the so-called stem cell niche consisting of fibroblasts, osteoblasts, and endothelial cells, governs the maintenance and mobilization of bone marrow stem cells (61,62,63). Mechanistically, cytokines inducing mobilization interfere with the interactions between stem cells and bone marrow stromal cells, which allow stem cells to disengage the bone marrow, and to pass through the sinusoidal endothelium to enter the blood stream. Stem cell mobilization is mediated by proteinases such as elastase, cathepsin G, and matrix metalloproteinases (MMPs) (64). A cytokine clinically used for the mobilization of CD34+ cells in patients is G-CSF, which releases the proteinases elastase and cathepsin G from neutrophils. These proteinases induce cleavage of adhesive bonds on stromal cells, which interact with integrins on hematopoietic stem cells (65). Moreover, these proteinases cleave the cytokine SDF-1, which is released by stromal cells and its receptor CXCR4 on stem and progenitor cells (66). Stem cell mobilization as a result of high levels of circulating SDF-1 appears to reverse the SDF-1 gradient across the bone marrow barrier, forcing CXCR-4+ cells to exit the bone marrow (67). However, VEGF, SDF-1, and placental growth factor (PlGF)-induced stem cell mobilization was shown to rely on MMP-9 (59,68). When PlGF is administered in the early phase of bone marrow recovery, it is chemoattractive

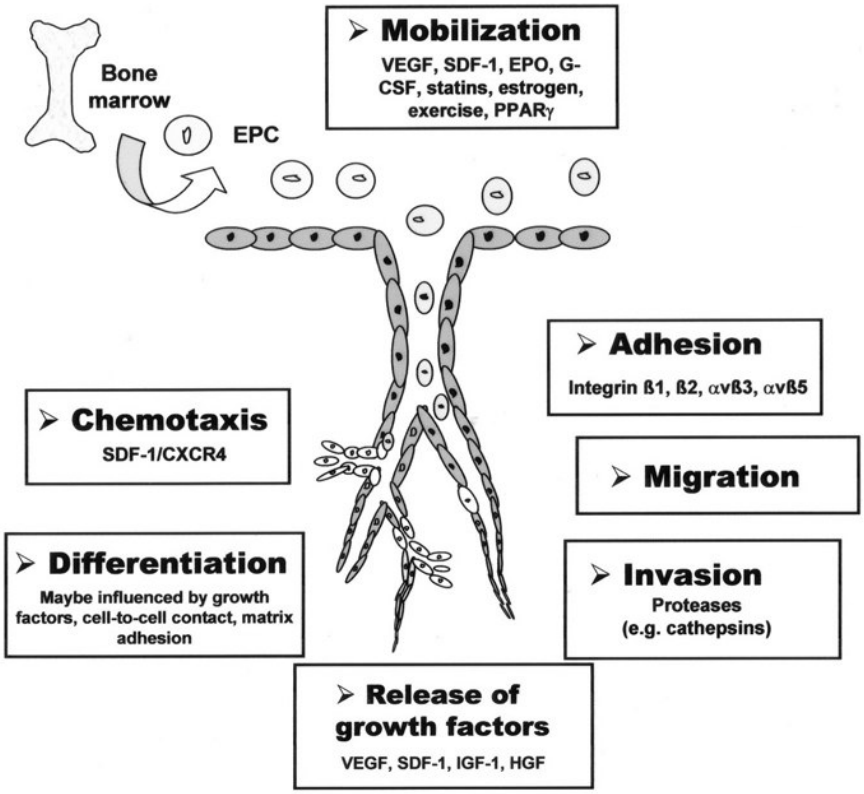

Figure 2. Mechanism of EPC homing and differentiation. Reprinted by permission from Wolters Kluwer Health, Circ. Res, Copyright (2004).

for VEGF-receptor-1+ stem cells, whereas in later stages PlGF functions are mediated by MMP-9 (68). Thus, increasing the local concentration of MMP-9 in the bone marrow cleaves membrane bound Kit ligand (mKitL) and, finally, releases soluble Kit ligand (KitL; also known as stem cell factor) (59). After all, this process transfers endothelial and hematopoietic progenitor cells from the quiescent to the proliferative niche. However, the question of whether G-CSF–induced stem cell mobilization depends on MMP-9 is still a matter of debate (59,69,70). This controversy might be explained by the fact that MMP-9 plays a pivotal role in growth factor-induced hematopoietic progenitor mobilization in wild-type animals, whereas compensatory upregulation of enzymes with a similar activity profile to MMP-9 might mask the impact of MMP-9 deficiency in the knockout model. As discussed, eNOS is essential to maintain adequate progenitor cell mobilization in response to distinct stimuli, including VEGF, statins, exercise, and estrogen, in the regulation of stem and progenitor cell mobilization (58,62,71,72). The defective mobilization was caused by the lack of eNOS (Nos3) provided by the bone marrow stromal microenvironment.

Therefore, eNOS deficiency in the bone marrow microenvironment impaired the mobilization of stem and progenitor cells from the bone marrow. In contrast, intravenous injection of stem and progenitor cells circumvented the defective mobilization from the bone marrow and improved the neovascularization after induction of hind limb ischemia. Therefore, eNOS-derived NO is a physiological regulator of stem and progenitor cell mobilization in the bone marrow stromal microenvironment.

Given that patients with coronary artery disease showed a diminished NO bioavailability in peripheral endothelial cells, one may speculate that this also translates into the bone marrow. EPC numbers are lower in patients with coronary artery disease or diabetes (55,74) and are correlated with NO-dependent vasorelaxation measured in the forearm (10).

Hypoxia, caused by an imbalance between oxygen supply and consumption, stimulates vasculogenesis in embryonic and adult tissue (75-79). Similarly, hypoxia, and the subsequent alteration of HIF-1, can induce VEGF and SDF-1, which stimulates migration of endothelial precursors during vasculogenesis (78,80). Therefore, it can be suggested that hypoxia

in embryonic and adult target tissue leads to an expression of chemotactic factors inducing migration of angioblasts and EPCs toward the region to be vascularized by vasculogenesis.

SDF-1 gene expression is regulated by the transcription factor hypoxia-inducible factor-1 in endothelial cells, resulting in selective in vivo expression of SDF-1 in ischemic tissue in direct proportion to reduced oxygen tension. HIF-1-induced SDF-1 expression increases the adhesion, migration and homing of circulating CXCR4-positive progenitor cells to ischemic tissue.

These data show that the recruitment of CXCR4-positive progenitor cells to regenerating tissues is mediated by hypoxic gradients via HIF-1-induced expression of SDF-1 (80).

In brief, the directed migration of angioblasts is regulated by a combination of different soluble and interaction molecules that build overlapping attractive and repulsive gradients of soluble factors forming the migratory route for the endothelial precursor cells. It can be suggested that the correct positioning of the endothelial cells after they start to migrate toward the target tissue is dependent on a fine balance of signals. Soluble factor from regions that should be vascularized diffuse to the endothelial precursors, attract them and keep them in their migratory phenotype state until they reach their final position (81).

Vascular endothelial growth factor caused the phosphorylation of AMPK, acetyl-coenzymeA (CoA) carboxylase (ACC), and eNOS in human cord blood-derived EPCs. The expression of EC markers, including VE-cadherin and intercellular adhesion molecule1 (ICAM-1), was also increased but blocked by Compound C, an AMPK inhibitor. AICAR, an AMPK agonist, increased the phosphorylation of ACC and eNOS and the expression of EC markers in a time- and dose-dependent manner, which reinforces the positive effect of AMPK on EPC differentiation.

The activation of eNOS by AMPK during EPC differentiation provides a novel mechanism for the pleiotropic effects of statins in benefiting the cardiovascular system (82).

There is accumulating evidence that platelets mediate the effect of hematopoietic cytokines to recruit bone marrow–derived cells to the vasculogenic niche. Thrombopoietic cytokines thrombopoietin (TPO) and stem cell factor (SCF) are significantly elevated shortly after ischemic tissue injury, and transgenic mice deficient in TPO or the TPO receptor (c-MPL) demonstrate significantly impaired hind – limb revascularization and inhibited angiogenic tumor growth (83).

Aggregation of activated platelets was necessary for the recruitment of bone marrow–derived c-Kit+ ScaI+ Lin- progenitor cells and CD34+ cells to sites of endothelial disruption (84). Hematopoietic cells were unable to adhere directly to the exposed extracellular matrix, but were

tethered to platelet surface P-selectin via P-selectin glycoprotein ligand (PSGL)-1 binding. Platelet release of SDF-1 provided an ongoing retention signal for bone marrow–derived cells at these sites (84). The mechanism by which platelets release SDF-1, VEGF and other growth factors in a site-specific fashion is likely to involve glycoprotein (GP) IIb– dependent platelet aggregation (84), inward receptor signaling, and MMP-9–mediated SDF-1 release (83). In addition to platelet-derived SDF-1, exposed smooth muscle cells also produce SDF-1 after arterial injury, and platelet activation via CXCR4 signaling leads to P-selection upregulation, further promoting arrest of bone marrow–derived cells to activated endothelium (85).

Taken together, these data suggest that aggregation and activation of platelets at sites of exposed subendothelium and vasculogenesis play a major role in the recruitment, differentiation, and incorporation of bone marrow–derived progenitor cells.

These insights have profound clinical implications; inhibition of platelet-deployed growth factors or their receptors may be an effective strategy to block tumor growth, whereas activation of these pathways may be used to accelerate revascularization and tissue regeneration (86).

Endothelial progenitor cells (EPCs) and hematopoietic progenitor cells are recruited to ischemic regions, improving neovascularization. β1 and β2 integrins play a crucial role for progenitor cell homing to ischemic tissues. Integrin activity is regulated by chemokines and their respective G protein– coupled receptors. The phosphatidylinositol-3-kinase catalytic subunit γ (PI3Kγ) is the PI3K isoform that selectively transduces signals from G protein–coupled receptors. A pharmacological PI3Kγ inhibitor significantly reduced chemokine – induced chemotaxis and stromal cell–derived factor (SDF)1α-induced transmigration of human EPCs. Moreover, the PI3Kγ inhibitor significantly reduced SDF1α-induced adhesion of EPCs to intercellular adhesion molecule-1 and human umbilical vein endothelial cell monolayers (87,88).

Adverse effects of EPC mobilization have been described as contribution of EPCs to tumor neovascularization in some tumor models (89). Moreover, circulating progenitor cells have been implicated in the neovascularization of arteriosclerotic lesions of allografts and in further atherosclerotic plaque progression in an ischemic setting (52,53). However, transfusion of EPCs enhanced re-endothelialization and reduced neointima formation after vascular injury (47). One may speculate that the endothelial repair capacity might override the potential harmful effects of plaque neovascularization. Thus, future studies have to determine the overall influence of EPC levels on atherosclerotic disease progression and prognosis (32).

Cardiovascular Risk Factors and EPCs

Small clinical studies have shown that the number of circulating EPCs inversely correlates with risk factors for atherosclerosis (10,55). Circulating CD34/KDR-positive progenitor cells are reduced to ~50% in patients with CAD compared with control groups. In addition, EPCs isolated from patients with CAD displayed an impaired migratory response, which was inversely correlated with the number of cardiovascular risk factors (55).

In patients with arterial hypertension, systolic blood pressure negatively correlates with the number of circulating CD133+ and CD34+/KDR+ EPCs, whereas the clonogenic potential (number of colony forming units–ECs) is not impaired by arterial hypertension (55). Experimental data demonstrate that angiotensin II, a potent mediator of detrimental effects in arterial hypertension, can accelerate the onset of EPC senescence by gp91 phox – mediated increase in oxidative stress, leading to an impaired proliferation activity of EPCs. Angiotensin II–induced EPC senescence was inhibited by treatment with the angiotensin II type 1 receptor blocker valsartan (90).

Recent studies have underlined the detrimental effects of types 1 and 2 diabetes on EPC function (74,91). Tepper et al demonstrated that in type 2 diabetes proliferation capacity of EPCs was reduced, adhesion capacity on activated human ECs was impaired.

Hypercholesterolemia was associated with reduced EPC numbers in 20 age-matched patients with hypercholesterolemia (8). Proliferative capacity, migratory activity, and in vitro vasculogenesis were negatively influenced by hypercholesterolemia. The underlying mechanisms are probably an increased rate of EPC senescence/apoptosis, as demonstrated after incubation of EPCs with oxidized LDL (92).

A role for HDL in promoting the repair of injured endothelium by stimulating the recruitment of endothelial progenitor cells (EPCs) into the endothelial layer (93).

Smoking has been identified as an important risk factor for reduced EPC numbers in one of the first studies on cardiovascular risk factors by Vasa et al (55). However, Wang et al recently demonstrated that the role of nicotine is more complex than initially expected (94). In an experimental study, they demonstrated that low concentrations of nicotine (10-8-10-12 mol/L) increased EPC number and activity, whereas higher (toxic) concentrations (>10-6 mol/L) were associated with cytotoxicity. In humans, Kondo et al demonstrated that chronic smokers (n=15) exhibit reduced EPC levels that can be restored after smoking cessation within 4 weeks (9).

In humans, a significant increase in progenitor cell numbers was observed in patients who resumed a standardized physical activity during a rehabilitation program (58), in patients with CAD (95), and in healthy individuals exercising for ≥30 minutes (96).

CRP, at concentrations known to predict adverse vascular outcomes, directly inhibits EPC differentiation, survival, and function, key components of angiogenesis and the response to chronic ischemia. This occurs in part via an effect of CRP to reduce EPC eNOS expression. The PPARγ agonist rosiglitazone inhibits the negative effects of CRP on EPC biology. The ability of CRP to inhibit EPC differentiation and survival may represent an important mechanism that further links inflammation to cardiovascular disease (97).

To contribute to tissue repair, EPCs, and stem cells in general, have to be equipped with antioxidative defense systems to survive in necrotic and ischemic tissues. Interestingly, a high resistance to oxidative stress has been considered a characteristic feature of stem cells (98,99).

He et al demonstrate that human EPCs possess a unique property to withstand oxidative injury and that elevated expression of manganese superoxide dismutase (MnSOD, a mitochondria-located SOD) is a critical intrinsic mechanism protecting EPCs against oxidative stress (100).

The finding that GPx-1 expression is essential for EPC functions may also have clinical implications, given that patients with chronic heart failure (101) and with type 2 diabetes (102) showed a downregulation of GPx-1. This in turn may contribute to the reduced EPC numbers and functions in patients with coronary artery disease and severe heart failure (55,103). However, other antioxidative enzymes such as superoxide dismutases and catalases are also downregulated in these patients (101,102). Thus, it seems mandatory to understand the specific role of the various antioxidative enzymes for EPC functions. In the future, a specific interference with the expression and activity of antioxidative enzymes in progenitor cells from patients might be a therapeutic strategy to improve their regenerative potential (104).

Schroeter et al (105) have now demonstrated that infusion of leptin-stimulated human EPCs reduces neointima formation and enhanced reendothelialization through upregulation of αvβ5- and α4-integrin–dependent adhesion to platelets in ferric chloride–induced vascular injury. With this elegant approach, the effect of leptin on EPC-mediated endothelial recovery can be readily discerned from leptin-enhanced thrombosis. This not only extends the mechanisms by which platelet-derived chemokines, P-selectin, and β2 integrins can support the arrest of EPCs and CD34+ progenitor cells at sites of arterial injury (85,106) but also adds a new dimension to the functional profile of leptin.

PPAR-δ activation with agonist (GW501516 or L-165041) increased the proliferation of human EPCs and protected them from hypoxia-induced apoptosis. In addition, PPAR-δ activation enhanced EPC functions, such as transendothelial migration, and tube formation. These actions by PPAR-δ activation in EPCs were dependent on the phosphatidylinositol 3-kinase/Akt pathway (107).

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 (108). In experimental 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 (109,110). 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 (58,71,109), under certain conditions, eNOS uncoupling may lead to an increased ROS production. To what extent the redox balance in stem cells (as opposed to cultured EPCs or crude bone marrow homogenates used in the study(111) favors such uncoupling processes is unclear and deserves further studies.

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-CFUs) in the bone marrow (11,55,112).

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 (113). 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 (114).

In summary, experimental and clinical studies demonstrated that aging interferes with progenitor cell functions. To what extent the dysfunction is exclusively related to age-associated telomere shortening and intrinsic cell dysfunction (eg, senescence) or might also involve age-dependent changes in paracrine activities remains to be defined (115).

EPCs Alteration in Diabetes

Both cytometric and culture methods have extensively demonstrated that type 1 and type 2 diabetic patients have less circulating EPCs than matched healthy subjects. Moreover, diabetic EPCs display functional impairment, such as reduced proliferation, adhesion, migration, and incorporation, into tubular structures (74,91,116). The mechanisms underlying EPC reduction in diabetes include weak bone marrow mobilization, decreased proliferation, and shortened survival in peripheral blood (127).

Poor collateral formation in diabetes may be attributed to weaker bone marrow stimulation from the ischemic tissue. Fadini et al (27) have recently confirmed this hypothesis, showing that bone marrow mobilization of EPCs after ischemia-reperfusion injury is defective in diabetic rats. Inability to mobilize EPCs was associated with downregulation of HIF-1 α and weakened release of marrow – stimulating factors, such as VEGF and SDF-1, ultimately leading to insufficient compensatory angiogenesis (15). Another study has shown that progenitor cell mobilization restored blood flow in diabetic mice (117). It is conceivable that HIF-1α deregulation in diabetes depends on an overproduction of reactive oxygen species (ROS).

Although molecular mechanisms that regulate EPC release in peripheral blood are complex and not fully understood, a role for the phosphatidylinositol (PI) 3-kinase/protein kinase-B and endothelial nitric oxide (NO) synthase pathways has been shown (72,118). As diabetes is characterized by altered activation of PI 3-kinase/Akt pathways and by reduced NO bioavailability (119), dysfunction of these subcellular pathways may be involved in the defective mobilization of EPCs from bone marrow. Hyperglycemia may be the common feature that affects survival and function of EPCs because similar alterations have been demonstrated in both type 1 and type 2 diabetes. In vivo, hyperglycemia induces oxidative stress by increasing the production of ROS and alters leukocyte and endothelial function (30).

Recently, Krankel et al. (120) have convincingly demonstrated that high glucose impairs proliferation, survival, and function of cultured EPCs, with concomitant-decreased NO production and matrix metalloproteinase-9 activity. Furthermore, activation of mitogen-activated protein kinases has been revealed as a potential mechanism of EPC dysfunction induced by high glucose (121). A definite demonstration is that correction of hyperglycemia by insulin therapy (15,122) can indeed restore the normal EPC pool.

Fadini et al (27) shown that patients with the metabolic syndrome have decreased levels of CD34+KDR+ EPCs compared with patients without the syndrome (116). Circulating CD34+ cells are synergically decreased by clustering components of the metabolic syndrome, and their levels negatively correlate with the homeostasis model assessment value, a measure of insulin resistance (123). In fact, insulin resistance, the typical hallmark of metabolic syndrome, is characterized by a defective activation of the PI3-kinase/Akt pathway and decreased endothelial NO synthase activity, which are considered essential for EPC mobilization and function. Oxidative stress plays a crucial role in the pathogenesis of diabetes complications (30), as well as in the entire atherogenic process. Therefore, stress-induced apoptosis may be one mechanism of EPC reduction in diabetes. The literature provides ample evidence that EPCs might decrease because of increased apoptosis and that EPCs are stress sensitive (124).

In summary, reduction in circulating EPCs in diabetic patients may recognize at least three pathophysiological explanations: impaired bone marrow mobilization, defective proliferation, and enhanced apoptosis. Remarkably, in accordance with Brownlee's unifying hypothesis (30), oxidative stress appears as a major determinant of all of these mechanisms. Interestingly, two very recent studies have demonstrated that the natural transketolase activator, benfotiamine, which is theoretically able to prevent the subcellular damage pathways triggered by oxidative stress, restored EPC-mediated healing of ischemic diabetic limbs in mice (125) and prevented hyperglycemia – mediated EPC dysfunction via modulation of the Akt pathway (126).

EPCs and the Diabetic Paradox

High blood glucose is an extremely detrimental factor for the retinal microvasculature. Hyperglycemic damage results in increased permeability, blood and serum leakage to the extravascular space, and progressive decline in retinal blood flow. Retinal ischemia and release of angiogenic factors stimulate the proliferation of microvessels, leading to proliferative retinopathy. Recently, intriguing novelties have been added to this pathogenetic model. In animals, bone

marrow–derived cells are mobilized and recruited at sites of retinal neovascularization in response to VEGF and SDF-1 (127,128). Therefore, not only local endothelial cells, but also EPCs may be involved in the development of proliferative retinopathy. This seems counterintuitive, as diabetes complications that may affect the same patient, such as diabetic retinopathy and PAD, can have opposing EPC alterations, the one being associated with increased and the other with decreased EPC levels. Parallely, a single diabetic patient can presentat the same time with complications of excessive angiogenesis (proliferative diabetic retinopathy) and of poor angiogenesis (symptomatic PAD) (27).

An unexplained paradox puzzles diabetologists: diabetic patients must face both poor vessel growth in ischemic heart and limbs and increased angiogenesis in retinal complications (129,130). Endothelial progenitor cells (EPCs) are marrow-derived cells involved in adult neovascularization and endothelial homeostasis (1,131). It has been postulated that low EPCs in peripheral blood may have a role in cardiovascular disease, and we have demonstrated that EPCs are reduced in macrovascular diabetes complications (10,116). On the other hand, an excess of EPCs may be involved in pathologic neoangiogenesis of cancer and proliferative retinopathy (132,133). Therefore, diabetes complications may be associated with both decreased and increased EPCs. Recently, novel therapeutic approaches have been directed to enrich the EPC pool in ischemic diseases and to block EPC function in proliferative diseases (128,134). These approaches in diabetic subjects require cautious evaluation of the implications carried by the paradox and new studies to unravel its causes (135,136).

Fadini et al (141) show that CD34+ cells and CD34+KDR+ cells are differentially altered in the presence of DR and PAD. These two common diabetes complications exhibit a very different behavior in terms of angiogenic response to ischemia, and this contrast has been termed "diabetic paradox." Many growth factors have been proposed to have a role in this phenomenon (137-139), all of which are potent stimuli for progenitor cell mobilization and homing to ischemic tissues (32). Indeed, recent data indicate that marrow-derived cells are involved in retinal neovascularization and that DR+ patients have increased levels of circulating progenitors (133,140). Conversely, macrovascular complications are characterized by reduced angiogenesis and exhausted EPC levels (116). This findings suggest that enhanced endothelial differentiation of circulating progenitors characterize DR, as shown by the high CD34+KDR+ proportion and the enhanced efficiency of EPC culture in contrast with the poor endothelial differentiation of PAD patients (141).

Therefore, the differential regulation of circulating progenitors, possibly in association with different oxygen gradients and local accumulation of growth factors, may explain why peripheral ischemia cannot stimulate angiogenesis as retinal ischemia does. Going deeper into the systemic events accompanying retinal vascular proliferation may provide novel therapeutic targets against peripheral ischemic complications. The notion that EPCs may be involved in retinal vascular proliferation should induce caution when trying to expand the EPC pool to ameliorate the cardiovascular profile. For instance, erythropoietin itself is an angiogenic factor that may worsen proliferative diabetic retinopathy (142).

Therapeutic Potential of EPC in Neovascularization

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 (143-146). 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 (147-149). 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(150).

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, includingVEGF 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 (151-153).

Clinical trials in patients with coronary artery disease or limb ischemia showed improvement after treatment with plasmid DNA encoding VEGF165 (154-157); thus VEGF may prove to be a feasible and successful therapy for vascular injury.

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 (158,159). 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, studies recently demonstrated that PDGF pathways are essential for maintaining the cardiomyogenic potential of Oct3/4+ bone marrow cells that is decreased with age (160).

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 (161). This protein is downregulated in the aging bone marrow and may also be depleted in the aging heart (161). 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 (59). SDF-1 has also been shown to promote bone marrow cell proliferation and angiogenesis (162).

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 (33). Similarly G-CSF also has stimulated EPC mobilization in clinical trials, but these EPCs display functional impairment of migratory properties in vitro.

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 EPCmediated 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 (46,56,163). 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 (164). Simvastatin activates the serine–threonine kinase Akt in endothelial cells, promoting endothelial cell survival and migration (165). Akt also acts downstream of VEGF and may therefore represent a key regulator of VEGF-mediated neovasculogenesis (166). 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 (167).

EPC Transplantation in Diabetes

Accelerated atherosclerosis is probably the most devastating among diabetes complications. The atherogenetic process in diabetic subjects is similar to that observed in their nondiabetic counterparts. However, diabetic vasculopathy is characterized by high prevalence, early development, bilaterality, rapid progression, and typical involvement of multiple distal sites. The severity of macrovascular complications in diabetes has been attributed to a profoundly impaired collateralization of vascular ischemic beds (168), which is insufficient to overcome the loss of blood flow, and leads to critical limb ischemia that often requires amputation. The mechanisms that hinder ischemia-induced neovascularization in diabetes had remained elusive until the discovery of EPCs. In animal models of diabetic vasculopathy, defective collateralization

was counteracted by administration of EPCs from control animals. Conversely, diabetic EPCs were not able to stimulate vascularization, even becoming antiangiogenic (169,170). Additionally, EPCs appeared important for vascularization and healing of diabetic wounds (171).

Patients with diabetes and PAD had a further significant decrease in circulating EPC levels, especially in the presence of ischemic foot lesions. Remarkably, EPC levels strongly correlated with the anklebrachial index, the most objective diagnostic and prognostic test for lower extremity arterial disease (116).

Lower-limb atherosclerosis: higher degrees of carotid stenosis, as well as worse stages of leg claudication and ischemic lesions, were associated with lower levels of EPCs, suggesting that EPC count may be considered a valuable marker of atherosclerotic involvement. Indeed, cytometric techniques, which allow EPC count, arewidely used for routine laboratory testing, and the determination of EPCs is sufficiently reproducible to be used in the clinical practice (11,40,123).

In the case that EPCs are defined as CD34+KDR+ cells. The clinical usefulness would stand in that EPCs not only mirror vascular function and atherosclerotic burden but also reflect the endogenous vasculoregenerative potential. Importantly, there are data suggesting that measuring EPCs would provide additional information over the classical risk factor analysis; in one study (11), CD34+KDR+ EPC count predicted cardiovascular events independently of risk factors and hard indexes, such as left ventricular ejection fraction. Moreover, EPCs isolated from diabetic patients with PAD exhibited impaired proliferation and adhesion to mature endothelium (172). We suggest that impaired collateralization leading to the clinical manifestations and complications of atherosclerosis in diabetes may be attributable to decreased and dysfunctional EPCs.

Huang et al. (23) have transplanted bone marrow–mobilized cells, as an EPC enriched fraction, into critically ischemic limbs of diabetic patients. Compared with standard therapy, cell therapy improved angiographic scores and ankle-brachial index values and reduced relevant end points, such as ulcer size and need for limb amputation (27). Recently, Körbling et al. were able to isolate a large quantity of clinically graded EPC from healthy volunteers by leukaparesis after G-CSF stimulation (173). This method may offer promise as a means of improving the efficacy of EPC transplantation for therapeutic neovascularization in the diabetic setting. However, previous data, while suggesting that G-CSF or granulocyte–macrophage colony – stimulating factor (GM-CSF) administration did increase EPC mobilization, the therapeutic effect in patients with coronary artery disease was conflicting (174,175).

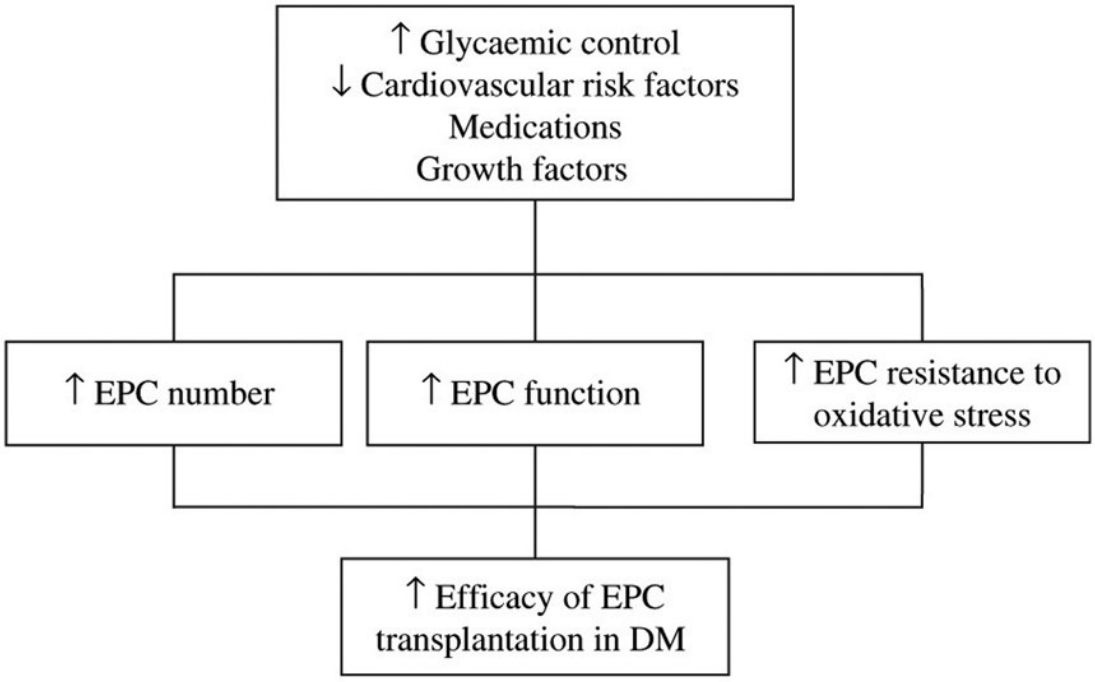

Figure 3. Approaches to improve efficacy of EPC transplantation in diabetic setting.
(Reprinted by permission from Wiley-Blackwell Publisher, Diabetes Obesity and Metabolism, Copyright 2007).

More recent data have shown that transplantation of G-CSF-mobilized EPCs for the treatment of critical limb ischaemia in patients with DM is safe and effective (23). Nevertheless, further studies are required to ensure the safety of this approach. EPC number can also be increased by augmenting the differentiation ability of MNC by co-administration of growth factors with unfractionated BM-MNC. For instance, co-administration of placental growth factor and BM-MNC into a diabetic hindlimb mouse model augments EPC differentiation from diabetic BM-MNCsin vitro (176).

Improved neovascularization can also be achieved in a diabetic hindlimb ischaemic mouse model by inhibition of NADPH oxidase–derived reactive oxidative species overproduction (177). Silencing of apoptotic gene expressionsuch as p53 renders EPCs derived from patients with T2DM more resistant to oxidative stress. This method also reduces EPC senescence and improves their ability to differentiate and form tubules in vitro (178). Lastly, administration of benfotiamine has been recently shown to increase EPC number and reverse EPC dysfunction in patients with DM (125,126).

Other growth factors such as VEGF, transforming growth factor-β1, angiopoetin-1, SDF-1, constitutive telomerase reverse transcriptase, hepatocyte growth factor, PDGF-B, basic fibroblast growth factor and medications such as erythropoietin, oestrogen, phosphodiesterase-5 inhibitor, puerarin and Ginkgo biloba have been shown to augment EPC number and function in a non-diabetic settings (179). These factors, however, have not been studied in the setting of diabetes but may be beneficial for therapeutic neovascularization in DM. The approaches to improve efficacy of EPC transplantation in diabetic setting is summarized in figure 3 (39).

Conclusion

Diabetes complications represent a huge burden for patients and health services. A common approach for the prevention and treatment of diabetes complications relies on the understanding of their complex pathophysiology.

During the last decade, data have become available indicating that alteration in EPCs may have an important causative role in all diabetes complications. New approaches such as EPC administration may represent novel treatments for diabetic vasculopathy in the future. To date, many barriers remain to such a therapeutic approach, hopefully in the near future these problems can be resolved.

References:

1. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964–967.
2. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci U S A 2000; 97: 3422–3427.
3. Kubota Y, Kishi K, Satoh H, Tanaka T, Nakajima H, Nakajima T. Transplanted endothelial progenitor cells augment the survival areas of rat dorsal flaps. Cell Transplant 2003; 12: 647–657.
4. Griese DP, Ehsan A, Melo LG, Kong D, Zhang L, Mann MJ, et al. Isolation and transplantation of autologous circulating endothelial cells into denuded vessels and prosthetic grafts: implications for cell-based vascular therapy. Circulation 2003; 108: 2710–2715.
5. He T, Smith LA, Harrington S, Nath KA, Caplice NM, Katusic ZS.. Transplantation of circulating endothelial progenitor cells restores endothelial function of denuded rabbit carotid arteries. Stroke 2004; 35: 2378–2384.
6. Takahashi M, Nakamura T, Toba T, Kajiwara N, Kato H, Shimizu Y.. Transplantation of endothelial progenitor cells into the lung to alleviate pulmonary hypertension in dogs. Tissue Eng 2004; 10: 771–779.
7. Imanishi T, Moriwaki C, Hano T, Nishio I. Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. J Hypertens 2005; 23: 1831–1837.
8. Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH, Zhu JH.. Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolaemia. Clin Sci (Lond) 2004; 107: 273–280.
9. Kondo T, Hayashi M, Takeshita K, Numaguchi Y, Kobayashi K, Iino S, et al. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. Arterioscler Thromb Vasc Biol. 2004; 24: 1442–1447.
10. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascularrisk. N Engl J Med 2003; 348: 593–600.
11. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med. 2005; 353: 999 –1007.
12. Schmidt-Lucke C, Rössig L, Fichtlscherer S, Vasa M, Britten M, Kämper U, et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. Circulation 2005; 111: 2981–2987.

13. Segal MS, Shah R, Afzal A, Perrault CM, Chang K, Schuler A, et al. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. Diabetes 2006; 55: 102–109.

14. Ii M, Takenaka H, Asai J, Ibusuki K, Mizukami Y, Maruyama K, et al. Endothelial progenitor thrombospondin-1 mediates diabetes-induced delay in reendothelialization following arterial injury. Circ Res 2006; 98: 697–704.

15. Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A, et al. Diabetes impairs progenitor cell mobilization after hindlimb ischemiareperfusion injury in rats. Diabetologia 2006; 49: 3075–3084.

16. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 2006; 367: 113–121.

17. Kang HJ, Lee HY, Na SH, Chang SA, Park KW, Kim HK, et al. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. Circulation 2006; 114: I145–I151.

18. Strauer BE, Brehm M, Zeus T, Köstering M, Hernandez A, Sorg RV, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002; 106: 1913–1918.

19. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, et al. Intracoronary autologous bonemarrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 2004; 364: 141–148.

20. Erbs S, Linke A, Adams V, Lenk K, Thiele H, Diederich KW, et al. Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebocontrolled study. Circ Res 2005; 97: 756–762.

21. Strauer BE, Brehm M, Zeus T, Bartsch T, Schannwell C, Antke C, et al. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. J Am Coll Cardiol 2005; 46: 1651–1658.

22. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 2003; 107: 2294–2302.

23. Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC.. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. Diabetes Care 2005; 28: 2155–2160.

24. Saigawa T, Kato K, Ozawa T, Toba K, Makiyama Y, Minagawa S, et al. Clinical application of bone marrow implantation in patients with arteriosclerosis obliterans, and the association between efficacy and the number of implanted bone marrow cells. Circ J 2004; 68: 1189–1193.

25. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 2002; 360: 427–435.

26. Tse HF, Thambar S, Kwong YL, Rowlings P, Bellamy G, McCrohon J, et al. Safety of catheter-based intramyocardial autologous bone marrow cells implantation for therapeutic angiogenesis. Am J Cardiol 2006; 98: 60–62.

27. Fadini MD, Sartore S, Agostini C, Avogaro A. Significance of Endothelial Progenitor Cells in Subjects With Diabetes. Diab Care 2007; 30: 1305 – 1313.

28. Akasaka T, Yoshida K, Hozumi T, Takagi T, Kaji S, Kawamoto T, et al. Retinopathy identifies marked restriction of coronary flow reserve in patients with diabetes mellitus. J Am Coll 1997; 30:935–941.

29. Klein BE, Klein R, McBride PE, Cruickshanks KJ, Palta M, Knudtson MD, et al. Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin Epidemiologic Study of Diabetic Retinopathy. Arch Intern Med 2004; 164:1917–1924.

30. Brownlee M. The pathobiology of diabetic complications: a unified mechanism. Diabetes 2005; 54:1615–1625.

31. Fadini GP, Agostini C, Avogaro A: Characterization of endothelial progenitor cells. Biochem Biophys Res Commun 2005; 336:1–2.

32. Aicher A, Zeiher AM, Dimmeler S: Mobilizing endothelial progenitor cells. Hypertension 2005; 45:321–325.

33. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, et al. Ischemia- and cytokineinduced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999; 5:434–438.

34. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005; 353:999–1007.

35. Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, et al. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood. 2000; 95: 3106–3112.

36. Handgretinger R, Gordon PR, Leimig T, Chen X, Buhring HJ, Niethammer D, et al. Biology and plasticity of CD133_ hematopoietic stem cells. Ann NY Acad Sci. 2003; 996: 141–151.

37. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. Blood. 1999; 93: 2951–2958.

38. Urbich C, Dimmeler S. Endothelial Progenitor Cells. Characterization and Role in Vascular Biology. Circ Res 2004; 95: 343 – 353.

39. Liew A, McDermott JH, Barry F, O'Brien T. Endothelial Progenitor Cells for the Treatment of Diabetic Vasculopathy: Panacea or Pandora's Box? Diabetes Obes Metab 2008; 10: 353 – 366.

40. Rustemeyer P, Wittkowski W, Jurk K, Koller A: Optimized flow cytometric analysis of endothelial progenitor cells in peripheral blood. J Immunoassay Immunochem 27: 77–88, 2006

41. Hur J, Yon CH, Kim HS, Choi JH, Kang HJ, Hwang KK, et al. Characterization of Two Types of Endothelial Progenitor Cells and Their Different Contributions to Neovasculogenesis. Arterioscler Thromb Vasc Biol 2004; 24: 288 – 293.

42. Yoon CH, Hur J, Park KW, Kim JH, Lee CS, Oh IY, et al. Synergistic Neovascularization by Mixed Transplantation of Early Endothelial Progenitor Cells and Late Outgrowth Endothelial Cells. The Role of Angiogenic Cytokines and Matrix Metalloproteinases. Circulation 2005; 112: 1618 – 1627.

43. Isner JM, Asahara T. Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. J Clin Invest. 1999; 103: 1231–1236.

44. Rehman J, Li J, Orschell CM, March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/ macrophages and secrete angiogenic growth factors. Circulation 2003; 107: 1164–1169.

45. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995; 1: 27–31.

46. Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, et al. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation 2002; 105: 3017–3024.

47. Werner N, Junk S, Laufs U, Link A, Walenta K, Bohm M, et al. Intravenous transfusion of endothelial progenitor cells reduces neointima formation after vascular injury. Circ Res 2003; 93: e17–e24.

48. Kaushal S, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW, et al. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. Nat Med 2001; 7: 1035–1040.

49. Hillebrands JL, Klatter FA, van Dijk WD, Rozing J. Bone marrow does not contribute substantially to endothelial-cell replacement in transplant arteriosclerosis. Nat Med 2002; 8: 194–195.

50. Griese DP, Ehsan A, Melo LG, Kong D, Zhang L, Mann MJ, et al. Isolation and transplantation of autologous circulating endothelial cells into denuded vessels and prosthetic grafts: implications for cell-based vascular therapy. Circulation 2003; 108: 2710–2715.

51. Fujiyama S, Amano K, Uehira K, Yoshida M, Nishiwaki Y, Nozawa Y, et al. Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial progenitor cells. Circ Res 2003; 93: 980–989.

52. Hu Y, Davison F, Zhang Z, Xu Q. Endothelial replacement and angiogenesis in arteriosclerotic lesions of allografts are contributed by circulating progenitor cells. Circulation 2003; 108: 3122–3127.

53. Silvestre JS, Gojova A, Brun V, Potteaux S, Esposito B, Duriez M, et al. Transplantation of bone marrow-derived mononuclear cells in ischemic apolipoprotein E-knockout mice accelerates atherosclerosis without altering plaque composition. Circulation 2003; 108: 2839–2842.

54. Rossig L, Dimmeler S, Zeiher AM. Apoptosis in the vascular wall and atherosclerosis. Basic Res Cardiol 2001; 96: 11–22.

55. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001; 89: e1–e7.

56. Llevadot J, Murasawa S, Kureishi Y, Uchida S, Masuda H, Kawamoto A, et al. HMG-CoA reductase inhibitor mobilizes bone marrow–derived endothelial progenitor cells. J Clin Invest 2001; 108: 399–405.

57. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation 2001; 103: 2885–2890.

58. Laufs U, Werner N, Link A, Endres M, Wassmann S, Jurgens K, et al. Physical Training Increases Endothelial Progenitor Cells, Inhibits Neointima Formation, and Enhances Angiogenesis. Circulation 2004; 109: 220–226.

59. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 2002; 109: 625–637.

60. Hristov M, Erl W, Weber PC. Endothelial Progenitor Cells. Mobilization, Differentiation, and Homing. Arterioscler Thromb Vasc Biol 2003; 23: 1185 – 1189.

61. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 2003; 425: 841– 846.

62. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell 1997; 88: 287–298.

63. Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature 2003; 425: 836–841.

64. Lapidot T, Petit I. Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp Hematol 2002; 30: 973–981.

65. Levesque JP, Takamatsu Y, Nilsson SK, Haylock DN, Simmons PJ. Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor. Blood 2001; 98: 1289–1297.

66. Levesque JP, Hendy J, Takamatsu Y, Simmons PJ, Bendall LJ. Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide. J Clin Invest 2003; 111: 187–196.

67. Hattori K, Heissig B, Tashiro K, Honjo T, Tateno M, Shieh Jhet al. Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. Blood 2001; 97: 3354–3360.

68. Hattori K, Heissig B, Wu Y, Dias S, Tejada R, Ferris B, et al. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. Nat Med 2002; 8: 841– 849.

69. Robinson SN, Pisarev VM, Chavez JM, Singh RK, Talmadge JE. Use of matrix metalloproteinase (MMP)-9 knockout mice demonstrates that MMP-9 activity is not absolutely required for G-CSF or Flt-3 ligandinduced hematopoietic progenitor cell mobilization or engraftment. Stem Cells 2003; 21: 417–427.

70. Levesque JP, Liu F, Simmons PJ, Betsuyaku T, Senior RM, Pham C, et al. Characterization of hematopoietic progenitor mobilization in protease-deficient mice. Blood 2004; 104: 65–72.

71. Landmesser U, Engberding N, Bahlmann FH, Schaefer A, Wiencke A, Heineke A, et al. Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. Circulation 2004; 110: 1933–1939.

72. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, et al. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 2003; 9: 1370 –1376.

73. Iwakura A, Luedemann C, Shastry S, Hanley A, Kearney M, Aikawa R, et al. Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. Circulation 2003; 108: 3115–3121.

74. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002; 106: 2781–2786.

75. Li B, Sharpe EE, Maupin AB, Teleron AA, Pyle AL, Carmeliet P, et al. VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. FASEB J 2006; 20: 1495–1497.

76. Gassmann M, Fandrey J, Bichet S, Wartenberg M, Marti HH, Bauer C, et al. Oxygen supply and oxygen-dependent gene expression in differentiating embryonic stem cells. Proc Natl Acad Sci U S A 1996; 93: 2867–2872.

77. Hoper J, Jahn H. Influence of environmental oxygen concentration on growth and vascular density of the area vasculosa in chick embryos. Int J Microcirc Clin Exp 1995; 15: 186 –192.

78. Ramirez-Bergeron DL, Runge A, Adelman DM, Gohil M, Simon MC. HIF-dependent hematopoietic factors regulate the development of the embryonic vasculature. Dev Cell 2006; 11: 81–92.

79. Yue X, Tomanek RJ. Effects of VEGF(165) and VEGF(121) on vasculogenesis and angiogenesis in cultured embryonic quail hearts. Am J Physiol 2001; 280: H2240–H2247.

80. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004; 10: 858–864.

81. Schmidt A, Brixius K, Bloch W. Endothelial Progenitor Cells Migration During Vasculogenesis. Circ Res 2007; 101: 125 – 136.

82. Li X, Han Y, Pang W, Li C, Xie X Shyy JYJ, et al. AMP-Activated Protein Kinase Promotes the Differentiation of Endothelial Progenitor Cells. Arterioscler Thromb Vasc Biol 2008; 28: 1789 – 1795.

83. Jin DK, Shido K, Kopp HG, Petit I, Shmelkov SV, Young LM, et al. Cytokine-mediated deployment of SDF-1 induces revascularization through recruitment of CXCR4+ hemangiocytes. Nat Med 2006; 12: 557–567.

84. Massberg S, Konrad I, Schurzinger K, Lorenz M, Schneider S, ZohlnhoeferD, et al. Platelets secrete stromal cell-derived factor 1_ and recruit bone marrow–derived progenitor cells to arterial thrombi in vivo. J Exp Med 2006; 203: 1221–1233.

85. Zernecke A, Schober A, Bot I, von Hundelshausen P, Liehn EA, Mopps B, et al. SDF-1alpha/CXCR4 axis is instrumental in neointimal hyperplasia and recruitment of smooth muscle progenitor cells. Circ Res 2005; 96: 784–791.

86. Rafii DC, Psaila B, Butler J, Jin DK, Lyden D. Regulation of Vasculogenesis by Platelet-Mediated Recruitment of Bone Marrow-Derived Cells. Arterioscler Thromb Vasc Biol 2008; 28: 217 – 222.

87. Chavakis E, Carmona G, Urbich C, Göttig S, Henschler R, Penninger JM. Phosphatidylinositol – 3 – Kinase - γ Is Integral to Homing Functions of Progenitor Cells. Circ Res 2008; 102: 942 – 949.

88. Mallat Z. PI3Kγ Helps an SDF Seeking Home . . . for EPCs. Circ Res 2008; 102: 871 – 872.

89. Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, et al. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 2001; 7: 1194 –1201.

90. Imanishi T, Hano T, Nishio I. Angiotensin II potentiates vascular endothelial growth factor-induced proliferation and network formation of endothelial progenitor cells. Hypertens Res 2004; 27: 101–108.

91. Loomans CJ, De Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, De Boer HC, et al. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes 2004; 53: 195–199.

92. Imanishi T, Hano T, Sawamura T, Nishio I. Oxidized low-density lipoprotein induces endothelial progenitor cell senescence, leading to cellular dysfunction. Clin Exp Pharmacol Physiol 2004; 31: 407– 413.

93. Tso C, Martinic G, Fan WH, Rogers C, Rye KA, Barter PJ. High – Density Lippoproteins Enhance Progenitor – Mediated Endothelium Repair in Mice. Arterioscler Thromb Vasc Biol 2006; 26: 1144 – 1149.

94. Wang X, Zhu J, Chen J, Shang Y. Effects of nicotine on the number and activity of circulating endothelial progenitor cells. J Clin Pharmacol 2004; 44: 881– 889.

95. Sandri M, Adams V, Gielen S, Linke A, Lenk K, Krankel Net al. Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes: results of 3 randomized studies. Circulation 2005; 111: 3391–3399.

96. Laufs U, Urhausen A, Werner N, Scharhag J, Heitz A, Kissner G, et al. Running exercise of different duration and intensity: effect on endothelial progenitor cells in healthy sub□ectsEur J Cardiovasc Prev Rehabil 2005; 12: 407– 414.

97. Verma S, Kuliszewski MA, Li SH, Szmitko PE, Zucco L, Wang CH, et al. C – Reactive Protein Attenuates Endothelial Progenitor Cell Survival, Differentiation, and Function. Further Evidence of a Mechanistic Link Between C – Reactive Protein and Cardiovascular Disease. Circulation 2004; 109: 2058 – 2067.

98. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. Science 2002; 298: 601–604.

99. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. □Stemness□transcriptional profiling of embryonic and adult stem cells. Science 2002; 298: 597–600.

100. He T, Peterson TE, Holmuhamedov EL, Terzic A, Caplice NM, Oberley LW, et al. Human Endothelial Progenitor Cells Tolerate Oxidative Stress Due to Intrinsically High Expression of Manganese Superoxide Dismutase. Arterioscler Thromb Vasc Biol 2004; 24: 2021 – 2027.

101. Linke A, Adams V, Schulze PC, Erbs S, Gielen S, Fiehn E, et al. Antioxidative effects of exercise training in patients with chronic heart failure: increase in radical scavenger enzyme activity in skeletal muscle. Circulation 2005; 111: 1763–1770.

102. Colak E, Ma□kic-SinghN, Stankovic S, Sreckovic-Dimitri□evic V, D□ord□evi□B, Lalic K, et al. Parameters of antioxidative defense in type 2 diabetic patients with cardiovascular complications. Ann Med 2005; 37: 613–620.

103. Valgimigli M, Rigolin GM, Fucili A, Porta MD, Soukhomovskaia O, Malagutti P, et al. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. Circulation 2004; 110: 1209–1212.

104. Haendeler J, Dimmeler S. Inseparably Tied. Functional and Antioxidative Capacity of Endothelial Progenitor Cells. Circ Res 2006; 98: 157 – 158.

105. Schroeter MR, Leifheit M, Sudholt P, Heida N-M, Dellas C, Rohm I, Alves et al. Leptin enhances the recruitment of endothelial progenitor cells into neointimal lesions after vascular in□uryby promoting integrinmediated adhesion. Circ Res 2008; 103: 536 –544.

106. Hristov M, Zernecke A, Bidzhekov K, Liehn EA, Shagdarsuren E, Ludwig A, et al. Importance of CXCR2 in the homing of human peripheral blood endothelial progenitor cells to sites of arterial in□uryCirc Res 2007; 100: 590 –597.

107. Han JK, Lee HS, Yang HM, Hur J, Jun SI, Kim JY, et al. Peroxisome Proliferator – Activated Receptor - δ Agonist Enhances Vasculogenesis by Regulating Endothelial Progenitor Cells Through Genomic and Nongenomic Activations of Phosphatidylinositol 3 – Kinase / Akt Pathway. Circulation 2008; 118: 1021 – 1033.

108. Thum T, Tsikas D, Stein S, Schultheiss M, Eigenthaler M, Anker SD, et al, Bauersachs J. Suppression of endothelial progenitor cells in human coronary artery disease by the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine. J Am Coll Cardiol 2005; 46: 1693–1701.

109. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, et al. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 2003; 9: 1370 –1376.

110. Sasaki K, Heeschen C, Aicher A, Ziebart T, Honold J, Urbich C, et al. Ex vivo pretreatment of bone marrow mononuclear cells with endothelial NO synthase enhancer AVE9488 enhances their functional activity for cell therapy. Proc Natl Acad Sci U S A 2006; 103: 14537–14541.

111. Thum T, Fraccarollo D, Schultheiss M, Froese S, Galuppo P, Widder JD, et al. Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. Diabetes 2007; 56: 666–674.

112. Kissel CK, Lehmann R, Assmus B, Aicher A, Honold J, Fischer-Rasokat U, et al. Selective functional exhaustion of hematopoietic progenitor cells in the bone marrow of patients with postinfarction heart failure. J Am Coll Cardiol 2007; 49: 2341–2349.

113. Blasco MA. Telomere length, stem cells and aging. Nat Chem Biol 2007; 3: 640–649.

114. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol 2007; 8: 703–713.

115. Dimmeler S, Leri A. Aging and Disease as Modifiers of Efficacy of Cell Therapy. Circ Res 2008; 102: 1319 – 1330.

116. Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, Grego F, et al. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. J Am Coll Cardiol 2005; 45: 1449–1457.

117. Jiao C, Fricker S, Schatteman G. The chemokine (C-X-C motif) receptor 4 inhibitor AMD3100 accelerates blood flow restoration in diabetic mice. Diabetologia 2006; 49:2786–2789.

118. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, et al. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J Clin Invest 2001; 108: 391–397.

119. Avogaro A, Fadini GP, Gallo A, Pagnin E, de Kreutzenberg S. Endothelial dysfunction in type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 2006; 16 (Suppl. 1):S39–S45.

120. Krankel N, Adams V, Linke A, Gielen S, Erbs S, Lenk K, et al. Hyperglycemia reduces survival and impairs function of circulating blood-derived progenitor cells. Arterioscler Thromb Vasc Biol 2005; 25: 698 –703.

121. Seeger FH, Haendeler J, Walter DH, Rochwalsky U, Reinhold J, Urbich C, et al. p38 mitogen-activated protein kinase downregulates endothelial progenitor cells. Circulation 2005; 111: 1184–1191.

122. Humpert PM, Neuwirth R, Battista MJ, Voronko O, von Eynatten M, Konrade I, et al. SDF-1 genotype influences insulin-dependent mobilization of adult progenitor cells in type 2 diabetes. Diabetes Care 2005; 28: 934–936.

123. Fadini GP, de Kreutzenberg SV, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A: Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. Eur Heart J 2006; 27:2247–2255.

124. Ingram DA, Krier TR, Mead LE, McGuire C, Prater DN, Bhavsar J, et al. Clonogenic endothelial progenitor cells are sensitive to oxidative stress. Stem Cells 2006; 25:297–304.

125. Gadau S, Emanueli C, Van Linthout S, Graiani G, Todaro M, Meloni M, et al. Benfotiamine accelerates the healing of ischaemic diabetic limbs in mice through protein kinase B/Akt-mediated potentiation of angiogenesis and inhibition of apoptosis. Diabetologia 2006; 49:405–420.

126. Marchetti V, Menghini R, Rizza S, Vivanti A, Feccia T, Lauro D, et al. Benfotiamine counteracts glucose toxicity effects on endothelial progenitor cell differentiation via Akt/FoxO signaling. Diabetes 2006; 55:2231–2237.

127. Csaky KG, Baffi JZ, Byrnes GA, Wolfe JD, Hilmer SC, Flippin J, et al. Recruitment of marrow-derived endothelial cells to experimental choroidal neovascularization by local expression of vascular endothelial growth factor. Exp Eye Res 2004; 78: 1107–1116.

128. Butler JM, Guthrie SM, Koc M, Afzal A, Caballero S, Brooks HL, et al. SDF-1 is both necessary and sufficient to promote proliferative retinopathy. J Clin Invest 2005; 115: 86–93.

129. Abaci A, Oguzhan A, Kahraman S, Eryol NK, Unal S, Arinc H, et al. Effect of diabetes mellitus on formation of coronary collateral vessels. Circulation 1999; 99: 2239–2242.

130. Ciulla TA, Amador AG, Zinman B. Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies. Diabetes Care 2003; 26:2653–2664.

131. Cho HJ, Kim HS, Lee MM, Kim DH, Yang HJ, Hur J, et al. Mobilized endothelial progenitor cells by granulocty-macropahge colony stimulatin factor accelerate reendothelization and reduce vascular inflammation after intravascular radiation. Circulation 2003; 108:2918–2925.

132. Peters BA, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC, et al. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nat Med 2005; 11:261–262.

133. Lee IG, Chae SL, Kim JC. Involvement of circulating endothelial progenitor cells and vasculogenic factors in the pathogenesis of DR. Eye 2006; 20: 546 – 552.

134. Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, et al. Transplantationof progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCAREAMI Trial. J Am Coll Cardiol 2005; 44:1690–1699.

135. Duh E, Aiello LP. Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox. Diabetes 1999; 48:1899–1906.

136. Fadini GP, Avogaro A. Autologous transplantation of granulocyte colony–stimulating factor–mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes (Letter). Diabetes Care 2006; 29:478–479.

137. Simo R, Lecube A, Garcia-Arumi J, Carrasco E, Hernandez C. Hepatocyte growth factor in the vitreous fluid of patients with proliferative diabetic retinopathy: its relationship with vascular endothelial growth factor and retinopathy activity. Diabetes Care 2004; 27:287–288.

138. Mitamura Y, Tashimo A, Nakamura Y, Tagawa H, Ohtsuka K, Mizue Y, et al. Vitreous levels of placenta growth factor and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Diabetes Care 2002; 25: 2352.

139. Freyberger H, Brocker M, Yakut H, Hammer J, Effert R, Schifferdecker E, et al. Increased levels of plateletderived growth factor in vitreous fluid of patients with proliferative diabetic retinopathy. Exp Clin Endocrinol Diabetes 2000; 108:106–109.

140. Grant MB, May WS, Caballero S, Brown GA, Guthrie SM, Mames RN, et al. Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization. Nat Med 2002; 8: 607–612.

141. Fadini GP, Sartore S, Baesso I, Lenzi M, Agostini C, Tiengo A, et al. Endothelial Progenitor Cells and the Diabetic Paradox. Diab Care 2006; 29: 714 – 716.

142. Watanabe D, Suzuma K, Matsui S, Kurimoto M, Kiryu J, Kita M, et al. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. N Engl J Med 2005; 353:782–792.

143. Shintani S, Murohara T, Ikeda H, Ueno T, Sasaki K, Duan J, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation. 2001; 103: 897–903.

144. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest. 2001; 107: 1395–1402.

145. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci U S A 2001; 98: 10344 –10349.

146. Ayach BB, Yoshimitsu M, Dawood F, Sun M, Arab S, Chen M, et al. Stem cell factor receptor induces progenitor and natural killer cell-mediated cardiac survival and repair after myocardial infarction. Proc Natl Acad Sci U S A.. 2006; 103: 2304 –2309.

147. Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, et al. Bone marrow-derived cells do not incorporate into the adult growing vasculature. Circ Res 2004; 94: 230 –238.

148. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res. 2004;94: 678–685.

149. Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S, Fuchs S, et al. Local delivery of marrow-derived stromal cells augmentscollateral perfusion through paracrine mechanisms. Circulation 2004; 109: 1543–1549.

150. Ballard VLT, Edelberg JM. Stem Cell and the Regeneration of the Aging Cardiovascular System. Circ Res 2007; 100: 1116 – 1127.

151. Ballard VL, Edelberg JM. Harnessing hormonal signaling for cardioprotection. Sci Aging Knowledge Environ 2005; 2005: re6.

152. Edelberg JM, Tang L, Hattori K, Lyden D, Rafii S. Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. Circ Res 2002; 90: e89–e93.

153. Qian HS, de Resende MM, Beausejour C, Huw LY, Liu P, Rubanyi GM, et al. Age-dependent acceleration of ischemic injury in endothelial nitric oxide synthase-deficient mice: potential role of impaired VEGF receptor 2 expression. J Cardiovasc Pharmacol 2006; 47: 587–593.

154. Losordo DW, Vale PR, Isner JM. Gene therapy for myocardial angiogenesis. Am Heart J 1999;138:S132–S141.

155. Symes JF, Losordo DW, Vale PR, Lathi KG, Esakof DD, Mayskiy M, et al. Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease. Ann Thorac Surg 1999; 68: 830–836.

156. Isner JM, Pieczek A, Schainfeld R, Blair R, Haley L, Asahara T, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. Lancet 1996; 348: 370 –374.

157. Baumgartner I, Pieczek A, Manor O, Blair R, Kearney M, Walsh K, et al. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. Circulation 1998;97:1114 –1123.

158. Xaymardan M, Tang L, Zagreda L, Pallante B, Zheng J, Chazen JL,et al. Platelet-derived growth factor-AB promotes the generation of adult bone marrow-derived cardiac myocytes. Circ Res. 2004; 94: e39–e45.

159. Edelberg JM, Lee SH, Kaur M, Tang L, Feirt NM, McCabe S, et al. Platelet-derived growth factor-AB limits the extent of myocardial infarction in a rat model: feasibility of restoring impaired angiogenic capacity in the aging heart. Circulation 2002; 105: 608–613.

160. Pallante BA, Duignan I, Okin D, Chin A, Bressan MC, Mikawa T, et al. Bone marrow Oct3/4+ cells differentiate into cardiac myocytes via age-dependent paracrine mechanisms. Circ Res 2007;100: e1–e11.

161. Ballard VL, Sharma A, Duignan I, Holm JM, Chin A, Choi R, et al. Vascular tenascin-C regulates cardiac endothelial phenotype and neovascularization. FASEB J 2006; 20: 717 –719.

162. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood. 2000; 95 : 952–958.

163. Werner N, Priller J, Laufs U, Endres M, Bohm M, Dirnagl U, et al. Bone marrow-derived progenitor cells modulate vascular reendothelialization and neointimal formation: effect of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition. Arterioscler Thromb Vasc Biol 2002; 22: 1567–1572.

164. Spyridopoulos I, Haendeler J, Urbich C, Brummendorf TH, Oh H, Schneider MD, et al. Statins enhance migratory capacity by upregulation of the telomere repeat-binding factor TRF2 in endothelial progenitor cells. Circulation 2004; 110: 3136 –3142.

165. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJet al. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat Med 2000; 6: 1004 –1010.

166. Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J Biol Chem 1998; 273: 30336 –30343.

167. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation 2002; 106: 3009 –3017.

168. Waltenberger J. Impaired collateral vessel development in diabetes: potential cellularmechanisms and therapeutic implications. Cardiovasc Res 2001; 49:554 –560.

169. Schatteman GC, Hanlon HD, Jiao C, Dodds SG, Christy BA. Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J Clin Invest 2000; 106: 571–578.

170. Awad O, Jiao C, Ma N, Dunnwald M, Schatteman GC. Obese diabetic mouse environment differentially affects primitive and monocytic endothelial cell progenitors. Stem Cells 2005; 23:575–583.

171. Asai J, Takenaka H, Kusano KF, Ii M, Luedemann C, Curry C, et al. Topical sonic hedgehog gene therapy accelerates wound healing in diabetes by enhancing endothelial progenitor cellmediated microvascular remodeling. Circulation 2006; 113:2413–2424.

172. Fadini GP, Sartore S, Albiero M, Baesso I, Murphy E, Menegolo M, et al. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. Arterioscler Thromb Vasc Biol 2006; 26: 2140–2146.

173. Körbling M, Reuben JM, Gao H, Lee BN, Harris DM, Cogdell D, et al. Recombinant human granulocytecolony-stimulating factor-mobilized and apheresiscollected endothelial progenitor cells: a novel blood cell component for therapeutic vasculogenesis. Transfusion 2006; 46: 1795–1802.

174. Zbinden S, Zbinden R, Meier P, Windecker S, Seiler C. Safety and efficacy of subcutaneous-only granulocytemacrophage colony-stimulating factor for collateral growth promotion in patients with coronary artery disease. J Am Coll Cardiol 2005; 46: 1636–1642.

175. Hill JM, Syed MA, Arai AE, Powell TM, Paul JD, Zalos G, et al. Outcomes and risks of granulocyte colonystimulating factor in patients with coronary artery disease. J Am Coll Cardiol 2005; 46: 1643–1648.

176. Tamarat R, Silvestre JS, Le Ricousse-Roussanne S, Barateau V, Lecomte-Raclet L, Clergue M, et al. Impairment in ischemia-induced neovascularization in diabetes: bone marrow mononuclear cell dysfunction and therapeutic potential of placenta growth factor treatment. Am J Pathol 2004; 164: 457–466.

177. Ebrahimian TG, Heymes C, You D, Blanc-Brude O, Mees B, Waeckel L, et al. NADPH oxidase-derived overproduction of reactive oxygen species impairs postischemic neovascularization in mice with type 1 diabetes. Am J Pathol 2006; 169: 719–728.

178. Rosso A, Balsamo A, Gambino R, Dentelli P, Falcioni R, Cassader M, et al. p53 Mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. J Biol Chem 2006; 281: 4339–4347.

179. Liew A, Barry F, O'Brien T. Endothelial progenitor cells: diagnostic and therapeutic considerations. Bioessays 2006; 28: 261–270.