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Clinical and therapeutical implications of EPC biology in atherosclerosis

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Abstract

Bone marrow-derived circulating endothelial progenitor cells have been successfully used to enhance angiogenesis after tissue ischemia. The role of endothelial progenitor cells in endothelial cell homeostasis and their putative role in atherogenesis have been recently investigated. Cardiovascular risk factors negatively influence endothelial progenitor cell number and function while vasculoprotection *e.g.* by statins, estrogens and physical activity may be partly mediated by progenitor cells. Endogenous mobilization or injection of *ex-vivo* generated endothelial progenitor cells is associated with an enhanced reendothelialization, an improvement of endothelial function and reduced atherosclerotic burden. In contrast, endothelial progenitor cells may promote plaque angiogenesis in animal models and may negatively influence plaque development and stability. However, in humans with coronary atherosclerotic disease, endothelial progenitor cells are a novel risk predictor for cardiovascular mortality and morbidity. In this review we focus on the role of circulating endothelial progenitor cells in endothelial cell repair mechanisms at the vascular wall and their potentially protective and therapeutic role in atherosclerotic disease.

Keywords: endothelium - endothelial progenitor cells - atheroslerosis

Introduction

Preventing atherosclerosis and cardiovascular disease is one of the most important issues worldwide with cardiovascular disease being the leading cause of death in the industrialized countries. The accumulation of cardiovascular risk factors including age, diabetes, hypertension, smoking and hyperlipidemia leads to the development of endothelial dysfunction, the first manifestation of atherosclerotic disease. This endothelial dysfunction is already present in healthy, asymptomatic humans and is char-

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acterized by an activation of endothelial cells (EC), decreased nitric oxide (NO) availability and constitutional changes at the cell's outer surface [1–3]. Ongoing deterioration of the endothelial monolayer finally leads to endothelial cell death, invasion of inflammatory cells and vascular smooth muscle cell proliferation. An initial functional impairment of the endothelial monolayer with a high chance of reversibility has turned into a structural damage. This damage of the endothelial cell layer is followed by the development of an atherosclerotic lesion unless sufficient repair mechanisms lead to a restoration of the endothelial monolayer.

The outcome in patients with atherosclerotic disease is mainly influenced by the cardiovascular consequences including myocardial infarction, congestive heart failure, stroke and peripheral artery disease. Therefore, therapeutic strategies for cardiovascular disease include the early prevention of endothelial cell death and endothelial dysfunction, the prevention of atherosclerotic plaque progression, and the effective therapy of myocardial infarction and congestive heart failure. Various therapeutic attempts using pharmacological agents have been made to positively influence and modulate vascular function and regenerate endothelial cells. Candidates include statins, angiotensin converting enzyme-inhibitors (ACE-I), and angiotensin II type 1 receptor blockers (ARB) which have all shown to mediate vasculoprotection independent of their primary action (*e.g.* lipid lowering or blood pressure control). The underlying molecular mechanisms of their vasculoprotective action remain to be determined.

Bone marrow (BM)-derived circulating endothelial progenitor cells (EPC) have been recently described as a population of pluripotent cells within the peripheral blood capable to differentiate into endothelial cell [4]. These cells have been successfully used to restore endothelial function and to enhance angiogenesis after tissue ischemia (Nickenig, unpublished data and [4–6]). Here we review the role of EPC in endothelial cell maintenance and their putative role in the prevention of atherosclerotic disease.

Endothelial progenitor cell biology

During embryogenesis, a close regional and functional development of peripheral blood and vascular wall cells is noticed and suggests the existence of a common origin, the putative hemangioblast. In 1997 Asahara *et al.* first described a circulating angioblast within human peripheral blood [4] which was able to differentiate *in vitro* into EC. These so-called EPC significantly contributed to neoangiogenesis after tissue ischemia *in vivo* [4, 7]. Meanwhile multiple studies have confirmed the pivotal role of EPC in tissue angiogenesis (for review see [8]). Currently, the definition of EPC is divergent and different cell population are termed EPC. A widely accepted consensus defines cells positive for the surface markers CD34 and Vascular Endothelial Growth Factor Receptor-2 (VEGFR2 or KDR in humans; flk-1 in mice) as EPC (Fig. 1). An immature subset of EPC express the surface marker CD133 [9–11]. The expression of CD34, VEGFR2 and CD133 is typically found on embryonic angioblasts and is a demonstration of the immature character of the cells. However, the expression of the mentioned surface markers does not necessarily define a single cell population and does not give insights about the origin of cells. It has been convincingly demonstrated that EPC bear various other cell surface markers including markers of the monocytic cell lineage. This heterogeneity in cell surface markers probably reflects different developmental stages of EPC during the maturational process from the BM residual cell to the mature vascular wall cell and may account for functional differences. We recently identified a CD34 negative, CD133 and VEGFR2 positive EPC subpopulation which functionally differs from CD34, CD133 and VEGFR2 positive EPC in terms of vasoregenerative capacity [12]. The CD34 negative, CD133 and VEGFR2 positive EPC subpopulation is a precursor of the CD34/CD133 positive EPC population and preferably homes to sites of ischemia (Fig. 1). In addition, unstable plaques of patients with acute coronary syndromes had significantly more CD34 negative, CD133 positive cells. Finally, in an experimental model of endothelial denudation the described cells had a higher reendothelialization potential compared to "conventional" EPC. The purpose of ongoing research is to determine not only the phenotypic characteristics of circulating EPC but also to further clarify the functional characteristics of these cells. *In vitro*, peripheral blood- or BM-derived mononuclear cells can be differentiated in an endothelial progenitor cell-like cell type characterized by the expression of lectin

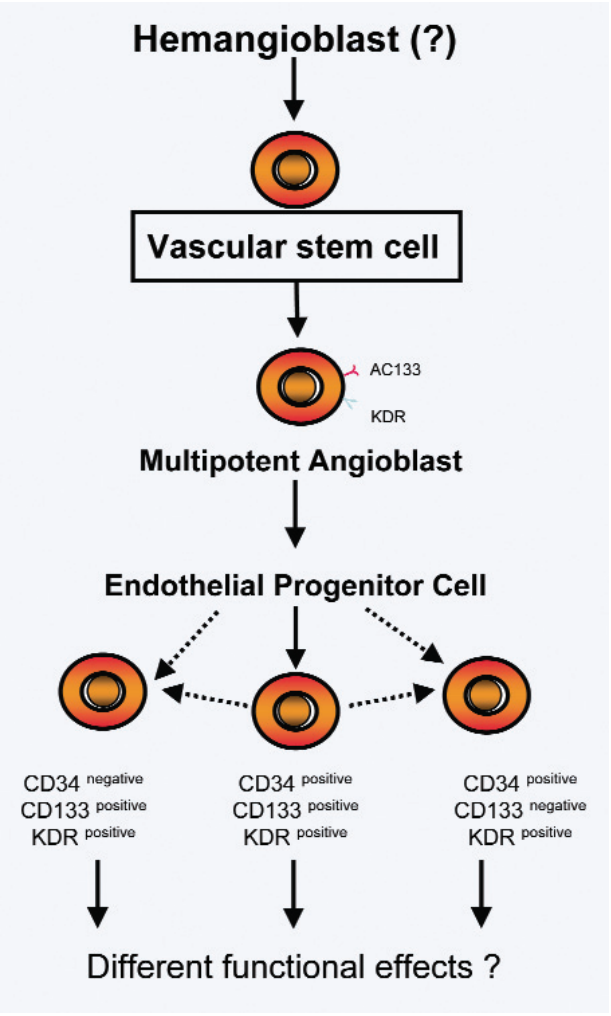


Fig. 1 Endothelial progenitor cells are putatively derived from the hemangioblast, circulate in peripheral blood and have the potential to differentiate into mature endothelial cells. The surface markers CD34, KDR (VEGFR2), CD133 define circulating endothelial progenitor cells. Recently, it has been demonstrated that different subpopulation display different functional activities concerning angiogenesis and endothelial cell repair. The CD34 negative, CD133 and VEGFR2 positive EPC subpopulation is a precursor of the CD34/CD133 positive EPC population and preferably homes to sites of ischemia.

and up-take of acetylated low-density lipoprotein (LDL) cholesterol [4, 13]. However, it is unclear at present whether these cells resemble the circulating "naïve" progenitor cells. In order to study the functional potential of progenitor cells, long-term cultures and the measurement of colony forming units (CFU-EC) have emerged as useful instruments. Again, these assays do not necessarily measure and reflect the situation in peripheral blood. The gold standard for the determination of circulating EPC remains the flow cytometry-based measurement of CD34/KDR and/or CD133-positive cells.

Clinical implications

The cardiovascular continuum

The development and progression of cardiovascular diseases is a multi-step process and can be regarded as a continuum of events. The presence of risk factors such as hyperlipidemia, hypertension, smoking or diabetes mellitus are major initial factors predisposing to the development of endothelial dysfunction and atherosclerosis. The progression to symptomatic coronary artery disease leads to myocardial ischemia and may be complicated after plaque rupture by myocardial infarction. Due to the improved medical treatment more people survive severe myocardial infarction and due to complex remodelling processes these patients might suffer from ventricular dilatation and congestive heart failure finally resulting in end-stage heart disease. It appears that the patient's level of circulating stem and progenitor cells influence each step of the cardiovascular continuum (Fig. 2). Here we will focus on vascular regeneration and the role and influence of stem and progenitor cells on initiation and progression of atherosclerotic lesions.

Cardiovascular risk factors influence EPC levels and function

The number of circulating EPC inversely correlates with risk factors for atherosclerosis [13, 14]. Compared to healthy controls, circulating CD34+/KDR+ progenitor cells are reduced to about 50% in patients with CAD. EPC isolated from patients with atherosclerotic disease show functional defects in migratory activity [13]. Systolic blood pressure has been shown to negatively correlate with the number of circulating CD133+ and CD34+/KDR+ EPC whereas the clonogenic potential measured as the number of CFU-EC is not impaired by arterial hypertension (Werner, unpub-

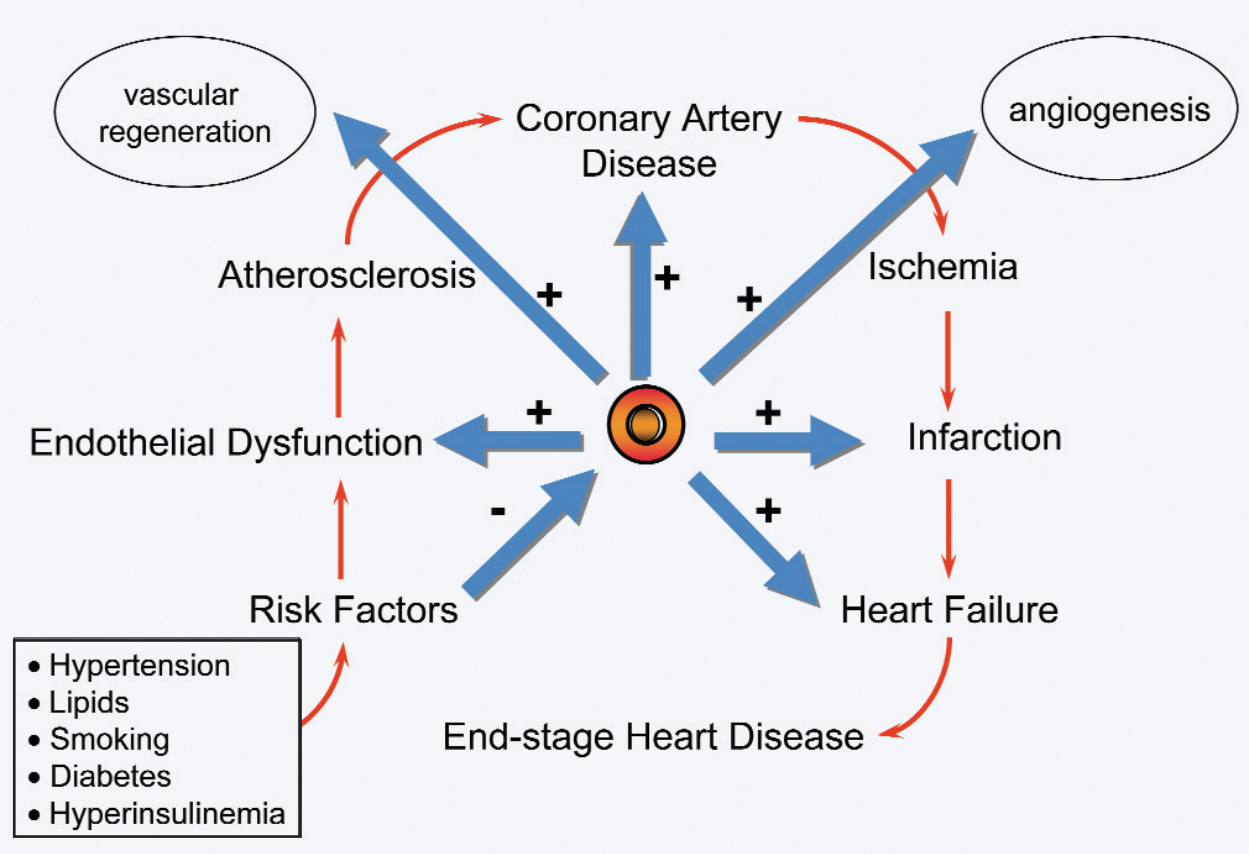


Fig. 2 Endothelial progenitor cells contribute to angiogenesis and endothelial cell repair in cardiovascular disease. These cells have been shown to be negatively influenced in number and function by cardiovascular risk factors, they contribute to endothelial cell repair in endothelial dysfunction, and inhibit progression of atherosclerotic lesions. Progenitor cells have been successfully used in therapeutic angiogenesis after myocardial infarction and congestive heart failure.

lished data and [13]). Apparently, angiotensin II (Ang II) accelerates the onset of EPC senescence by a gp91 phox-mediated increase in oxidative stress leading to an impaired proliferation activity of EPC. Treatment with the AT1 receptor blocker (ARB) valsartan or the ACE-I ramipril can neutralize these negative effects [15, 16] resulting in increased EPC levels. Experimental and clinical studies have convincingly described detrimental effects of diabetes on EPC number and function [17, 18]. In type II diabetes EPC proliferation is reduced, adhesion impaired, and diabetic EPC show reduced tube formation ability *in vitro*. Hyperglycemia was identified to mediate the detrimental effects on EPC by a decrease in nitric oxide (NO) production and metalloproteinase-9 (MMP-9) activity explaining the negative association between haemoglobin A1c (HbA1c) with progenitor cell levels [19]. But there is hope for diabetic patients: Placenta growth factor (PlGF) is able to increase EPC differentiation from diabetic BM cells by sixfold and glitazones have been shown to positively influence EPC biology [20, 21]. In addition, 40mg olmesartan increased circulating EPC counts in a prospective, double-blind study in 18 patients with type II diabetes [22]. Few studies have investigated the influence of LDL-C [23–26] on EPC number and function. Hypercholesterolemia is associated with reduced EPC levels, decreased proliferative capacity, migratory activity and *in vitro* vasculogenesis [23]. These negative effects can be abrogated by HMG-CoA reductase inhibitors (statins). Statins increase EPC number and function in a PI 3-kinase/Akt dependent pathway [27]. Smoking is an important risk factor for atherosclerosis and has been associated with decreased EPC numbers [13]. Chronic smok-

ers have reduced EPC levels which can recover after smoking cessation within 4 weeks [28]. Wang *et al.* recently demonstrated that nicotine may be a two-edged sword [29]. Low concentrations of nicotine increased EPC levels while higher concentrations (>10^{-6} mol/L) were associated with decreased EPC levels. Various other cardiovascular risk factors (*e.g.* homocysteine [30], C-reactive protein (CRP) [31, 32]) are associated with reduced EPC numbers and function. CRP is an important marker of inflammation and associated with endothelial dysfunction and atherosclerosis. EPC numbers *in vitro* are significantly reduced compared to controls when incubated with CRP and endothelial surface markers (*e.g.* lectin, VE-cadherin) vanish [32]. *In vitro* angiogenesis is significantly impaired in the presence of CRP and can be antagonized by co-treatment with rosiglitazone. In some instances, not only the presence of risk factors but the absence of vasculoprotective agents negatively influences EPC levels. Physical inactivity is associated with increased oxidative stress, endothelial dysfunction, and atherosclerosis in experimental models [33]. Mice subjected to regular physical activity show higher EPC levels compared to mice subjected to a sedentary lifestyle [34]. In patients and healthy subjects regular physical activity can upregulate EPC levels underlining the detrimental action of physical inactivity [34–36]. A similar mechanism has been observed for estrogens. Estrogen deficiency after ovariectomy is associated with reduced EPC counts and a impaired endothelial cell rejuvenation [37]. Estrogen substitution on the other hand completely normalizes EPC counts and restores the endothelium after experimentally induced EC damage [37].

EPC and reendothelialization

The number of CFU-EC *in vitro* is a predictor for endothelial function in healthy subjects without clinical signs of atherosclerosis [14]. In patients with manifest atherosclerotic disease the number of circulating EPC is significantly reduced [4, 13]. These observations raised the question whether atherosclerotic disease may be significantly influenced by circulating EPC. Two scenarios are possible: **1.** Circulating EPC contribute to endothelial repair mechanisms at the vascular wall thereby preventing the initiation and/or progression of atherosclerotic disease. In this case, the lack of a sufficient number of circulating EPC in patients with atherosclerotic disease would be a contributing cause for the presence of atherosclerotic lesions. **2.** The decrease of EPC is an epiphenomenon and not causative for the development of atherosclerotic disease.

In order to elucidate the underlying mechanisms of EPC in endothelial cell regeneration and atherogenesis various animal models have been evaluated. The systemic transfusion of *ex-vivo* expanded EPC can enhance reendothelialization after focal endothelial cell damage in a mouse model of endothelial denudation. Interestingly, not only the systemic transfusion of stem and progenitor cells but also endogenous mobilization of the organism´s own stem cell pool is associated with an enhancement of reendothelialization in different models of endothelial denudation (Fig. 3). The effect of recombinant human G-CSF on neointimal formation was evaluated in a balloon injury model in the rat carotid artery. Neointimal formation was markedly attenuated by G-CSF treatment (39% versus the control; $P<0.05$) due to an enhancement of re-endothelialization (1.8-fold increase *vs.* control; $P<0.05$)[38]. Regenerated endothelium was functionally intact as demonstrated by NO-dependent vasodilatation. Similar results have been shown by our group and others in mice using a statin-based mobilization of stem and progenitor cells [39, 40]. Using GFP chimeras we could demonstrate that indeed the endogenous progenitor cells pool contributed to the restoration of the endothelium after focal wire-induced endothelial denudation[39].

To further elucidate the vasculoprotective role of progenitor cells in humans, various studies have been performed evaluating the role of EPC in human endothelial cell regeneration. George *et al.* investigated the role of EPC in patients with in-stent restenosis [41]. They demonstrated that reduced numbers of circulating EPC in patients with diffuse in-stent restenosis may contribute to the excessive proliferative process in these patients. Furthermore, fibronectin-binding was significantly reduced in patients with in-stent restenosis as compared with patients with patent stents indicating a protective role of progenitor cells after endothelial denudation due to percutanous coronary interventions. Clinical implications may be enor-

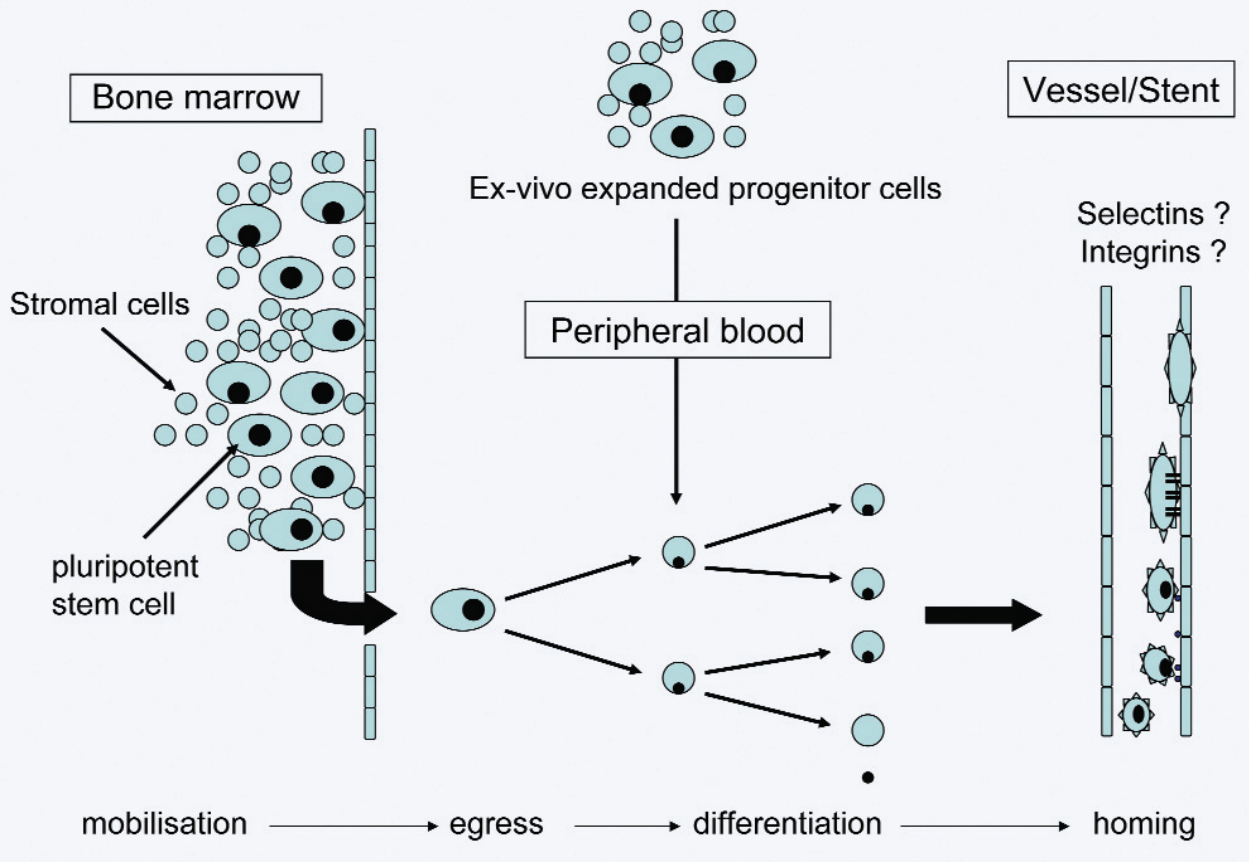


Fig. 3 Systemic transfusion of stem and progenitor cells and endogenous mobilization of the organism´s own stem cell pool is associated with an enhancement of reendothelialization of the injured vessel wall.

mous: A rapid reendothelialization of stents by EPC may prevent in-stent restenosis and potentially also early stent thrombosis. A first major study to evaluate whether rapid reendothelialization of stainless steel stents may prevent stent thrombosis and reduce restenosis was published recently [42]. Sixteen patients with *de novo* CAD were treated with "EPC-capturing" anti-CD34 coated stents in this safety study. The composite major adverse cardiac and cerebrovascular events (MACCE) rate was 6.3% after nine month due to a target vessel revascularization in a single patient. The mean angiographic late luminal loss was 0.63 +/- 0.52 mm, and percent stent volume obstruction by intravascular ultrasound analysis was 27.2 +/- 20.9% after 6 month. In addition, studies using stents seeded with EPC have been evaluated [43, 44]. Further studies will need to demonstrate the efficacy of the described approaches.

EPC and atherosclerosis

If EPC contribute to an enhanced endothelial cell repair after focal endothelial cell damage, EPC may have a pivotal role in maintaining the integrity of the endothelium in conditions of a dissiminating endothelial cell damage as seen for example in endothelial dysfunction. Endothelial dysfunction is the earliest manifestation of atherosclerotic disease and is characterized by reduced nitric oxide bioavailability and, on the cellular level, on a progressive loss of endothelial cells [45]. The systemic transfusion of EPC in hypercholesterolemic ApoE-knock-out mice significantly improves endothelial dysfunction as demonstrated in organ chamber experiments (Wassmann, personal communication). In a key publication, Rauscher and colleagues demonstrated that the systemic transfusion of stem and progenitor cells derived from young non-atherosclerotic ApoE-/- mice prevents atheroscle-

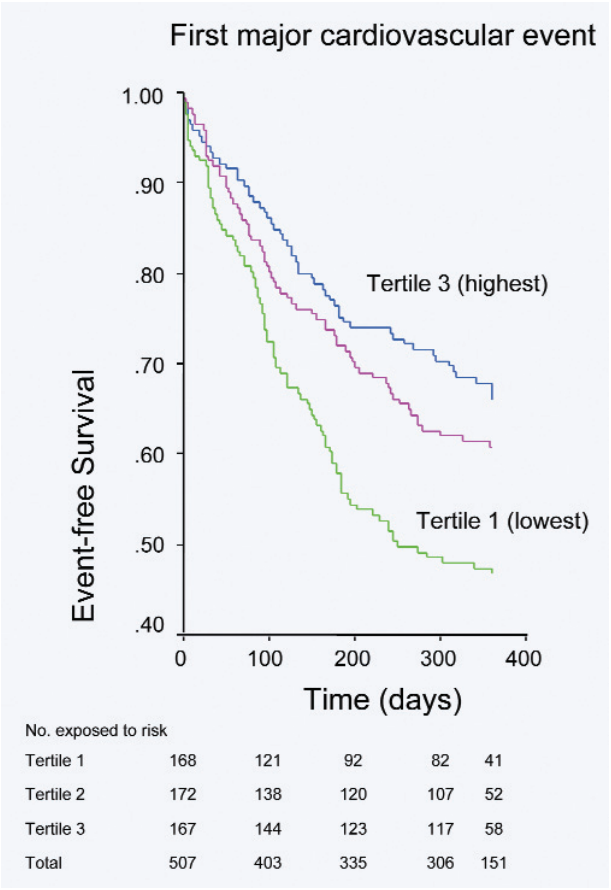
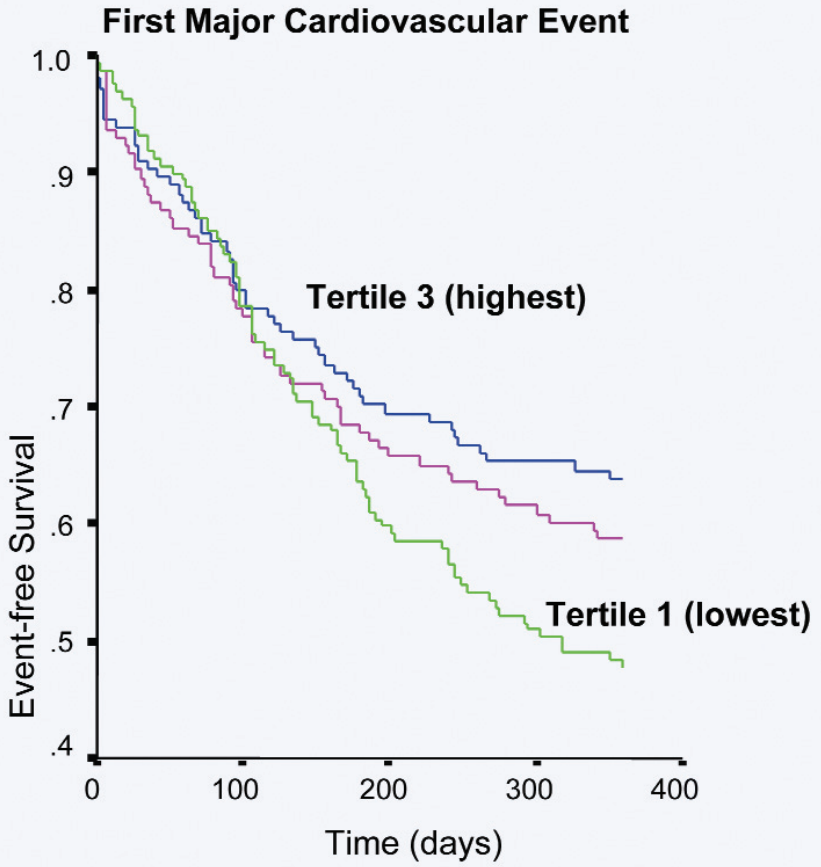


Fig. 4a The *Endothelial Progenitor Cells in Coronary Artery Disease* (EPCAD) study measured the number of CD34+/KDR+ EPC in 519 patients with angiographically documented coronary artery disease and correlated with cardiovascular outcomes [50]. The cumulative event-free survival increased stepwise across tertiles of baseline EPC levels for the occurrence of a first major cardiovascular event. Multivariate analysis identified EPC as an independent predictor for cardiovascular death, first major cardiovascular event, revascularization, and hospitalization.

rotic lesion progression in ApoE-/- recipients despite persistent hypercholesterolemia [46]. Treatment with bone marrow stem cells from aged ApoE-/- mice with manifest atherosclerosis did not effectively prevent atherosclerotic lesion progression. Apparently, endothelial cell repair capacity depends on the age of stem cells, underlining the important influence of cardiovascular risk factors on the bone marrow. Based on these findings, the same group investigated specific gene expression patterns in various disease states in murine atherosclerosis using a microarray-based approach and tried to determine the point in disease progression at which endothelial cell repair by stem cells ceased to be efficient [47]. The gene profile of early atherosclerotic disease was very similar to the gene profile seen in moderately diseased mice treated with stem cells from young mice while the gene profile of moderate atherosclerotic disease was similar to aortas treated with stem cells from old mice (>1 year of age). The authors demonstrated that the loss of competent rejuvenation was paralleled with the initiation of atherosclerotic lesions. These experimental results provide strong evidence for the vasculoprotective action of stem and progenitor cells at least in animal models.

Evidence that BM-derived EPC contribute to endothelial cell regeneration in humans comes from computer-based simulation models. In these simulation models, the maintenance of the endothelial monolayer by division of adjacent EC and the replacement of apoptotic EC by EPC homing was simulated [48]. Under physiological conditions, the integrity of the endothelial monolayer can be maintained by replication of adjacent cells. However, in conditions of oxidative stress due to aging, damage of the integrity of the endothelium was prevented by progenitor cell homing. A homing rate of 5% per year was sufficient to significantly delay defects in endothelial integrity. A similar model demonstrated the contribution of EPC to tumor neoangiogenesis [49]. Together with the experimental findings these computer simulations underline the pivotal role of EPC in vessel wall homeostasis. First evidence that EPC have a vasculoprotective action in patients with atherosclerotic disease comes from the recently published *Endothelial Progenitor Cells in Coronary Artery Disease* (EPCAD) study. The number of CD34+/KDR+ EPC was measured in 519 patients with angiographically documented CAD and correlated with cardiovascular outcomes [50]. Primary endpoints included cardiovascular mortality, the occurrence of a first major cardiovascular event (myocardial infarction, hospitalization, revascularization, and cardiovascular death), revascularization, hospitalization, and all-cause mortality after 12 months. The cumulative event-free survival increased stepwise across tertiles of baseline EPC levels for cardiovascular mortality, first major cardiovascular event, revascularization, and hospitalization (Fig. 4a). After adjustment for vascular risk factors, drug therapy, and concomitant disease, increased EPC

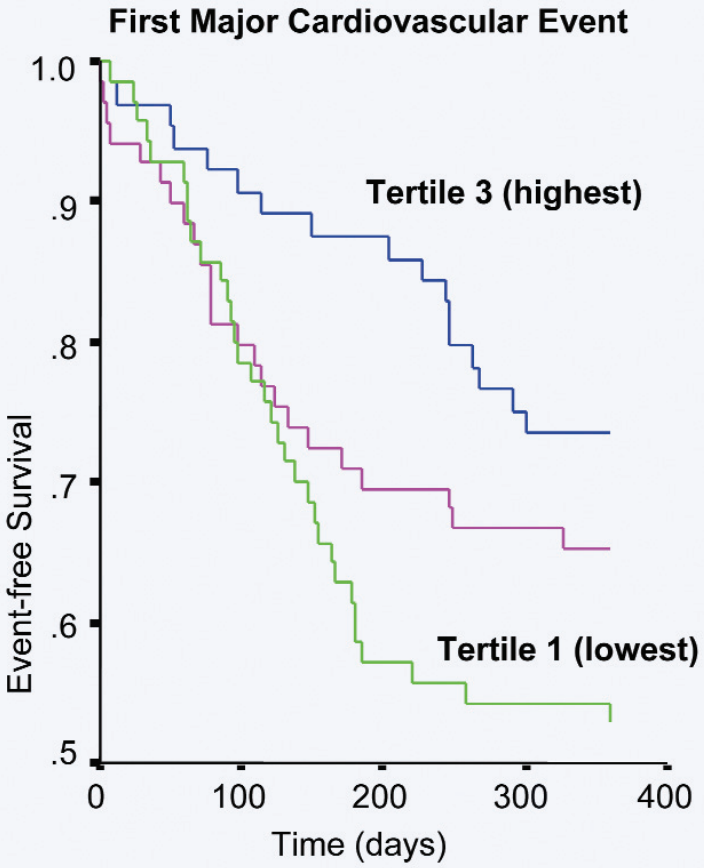


Outcome	P-value (log Rank)
Cardiovascular Death	0.035
First Major Cardiovascular Event	0.045
Revascularization	0.499
Hospitalization	0.044
Death from any Cause	0.196

$CD133^+$ Endothelial Progenitor Cells and Outcome.

Fig. 4b In addition to the number of CD34+/KDR+ EPC, the number of CD133+ EPC was also predictive for cardiovascular outcome in the EPCAD study, underlining the importance of these cells in cardiovascular disease.

levels were independently associated with a lower risk for cardiovascular death (hazard ratio (HR) 0.31 (95 percent confidence interval (CI) 0.16-0.63, p=0.001), first major cardiovascular event (HR 0.74 (95 percent CI 0.62-0.89, p=0.002), revascularization (HR 0.77 (95 percent CI 0.62-0.95, p=0.017), and hospitalization (HR 0.76 (95 percent CI 0.63-0.94, p=0.012). Interestingly, these results were confirmed when correlating the CD133+ EPC sub-fraction with cardiovascular outcome (Fig. 4b). In a subgroup of patients the number of colony forming units endothelial cells (CFU-EC) as a marker of the clonogenic potential of formerly circulating EPC were determined and in addition to the number of circulating cells the functional capacity closely correlated with cardiovascular event rates (Fig. 4c) [50].



Outcome	P-value (log Rank)
Cardiovascular Death	0.514
First Major Cardiovascular Event	0.034
Revascularization	0.012
Hospitalization	0.014
Death from any Cause	0.852

Number of Colony Forming Units – Endothelial Cells and Outcome.

Fig. 4c In a subgroup of patients in the EPCAD study, the number of colony forming units endothelial cells (CFU-EC) as a marker of the clonogenic potential of formerly circulating EPC was determined. In addition to the number of circulating cells the functional capacity closely correlated with cardiovascular event rates.

Plaque development and angiogenesis

Inhibition of plaque angiogenesis has been associated with beneficial effects on plaque growth and stability. Moulton *et al.* demonstrated that the density of vasa vasorum correlates with the extent of inflammatory cells but not the size of atheromas in ApoE-/- mice [51]. Microvessels at the base of the plaque have been associated with a higher risk for plaque rupture [52]. Since EPC play an important role in neoangiogenesis, these cells may promote plaque angiogenesis and may have a negative impact on plaque development and stability. In a study by Hu *et al.* neointimal lesions in allografts contained endothelial cells derived from circulating progenitor cells. The authors postulated a role of bone marrow-

derived cells in transplant arteriosclerosis [53]. A similar role of EPC has been postulated not only in plaque angiogenesis but also in tumor angiogenesis [54–57], and diabetic retinopathy [58]. However, there is convincing evidence from a number of studies that stem and progenitor cells have protective effects in atherosclerosis by contributing efficiently to endothelial cell repair mechanisms. So how can we solve the discrepancy concerning atherosclerosis? First of all, the role of EPC has not been convincingly investigated in plaque angiogenesis so the exact role of EPC in this specific context is unknown. Secondly, it is known from other conditions that angiogenesis can be a two-edged sword in the same disease: retinal angiogenesis is accelerated in diabetes and in the same disease wound and tissue angiogenesis *e.g.* after ischemia is impaired. Therefore, the role of EPC in plaque angiogenesis needs to be further defined [59].

Therapeutical implications

Given the experimental and clinical results concerning the role of stem and progenitor cells in maintenance of the endothelium´s integrity, a therapeutic approach using progenitor cells for the prevention of atherosclerotic disease seems reasonable. Two main requisites need to be accomplished to effectively use stem cells for therapy in atherosclerotic disease: **1.** We need to know the actual degree of endothelial cell damage in order to evaluate vascular injury and to monitor therapeutic effects. **2.** It is crucial to get a status of the regenerative capacity of the organism.

The above mentioned study by Karra *et al.* [47] clearly demonstrates that at a distinct point in atherosclerotic development (at least on the genomic level) the physiological balance between endothelial cell damage and endothelial cell regeneration which is assumed in healthy, non-atherosclerotic patients destabilizes and damaging forces overwhelm regeneration resulting in the initiation and progression of an atherosclerotic lesion. Endothelial cell damage can be measured *in vivo* using circulating endothelial microparticles (EMP) [60, 61]. These membrane vesicles are shed from activated and apoptotic endothelial cells and can be quantified using flow cytometry. EMP are increased in all conditions of systemic endothelial cell damage *e.g.* arterial hypertension, diabetes, hyperlipidemia as well as in acute coronary syndromes [62–65]. EMP have been shown to correlate with endothelial function *in vitro* [60]. Furthermore, circulating EMP significantly correlate with the degree of coronary endothelial function in patients with coronary artery disease [66].

So what are the therapeutic consequences? In the early stages of atherosclerotic disease *e.g.* at the level of endothelial dysfunction, risk factor modifications are apparently the therapeutical key solution. Risk factor reduction reduces the direct negative impact on the vascular wall and positively influences progenitor cell number and function. According to the INTERHEART study [67] nine easily measurable cardiovascular risk factors are associated with more than 90% of the risk of an acute myocardial infarction in a large global case-control study [67]. Accumulation of various risk factors (smoking, hypertension, and diabetes) increased the odds ratio for acute myocardial infarction to 13.01 (99% CI 10.69–15.83) compared to patients without a comparable risk profile. Although, the correlation between risk factors and atherosclerosis and resulting myocardial infarction is well known, compliance with life style modifications and risk factor reduction is poor questioning a risk factor modifying therapeutic approach.

In advanced stages of atherosclerotic disease namely at that point when regeneration is impaired and the endothelium´s integrity can no longer be reconstituted, strengthening of the organism´s regenerative capacity may be an additive strategy in addition to a single risk factor modification strategy (Fig. 5). The problem is: How to strengthen regeneration? Cell-based therapies widely used for example after myocardial infarctions are not applicable for a systemic disease like atherosclerosis. Currently, therapeutic approaches using mobilizing agents such as erythropoietin are promising due to their dual effects: increasing number of peripheral blood circulating EPC and improving cell function of risk factor-damaged cells at the same time. Erythropoietin treatment increases number, proliferation, and migration of mouse embryonic bodies, increases the formation of endothelial cells from embryonic bodies and human EPC [68]. However, we are still in the process of searching for the “ideal” substance. Various

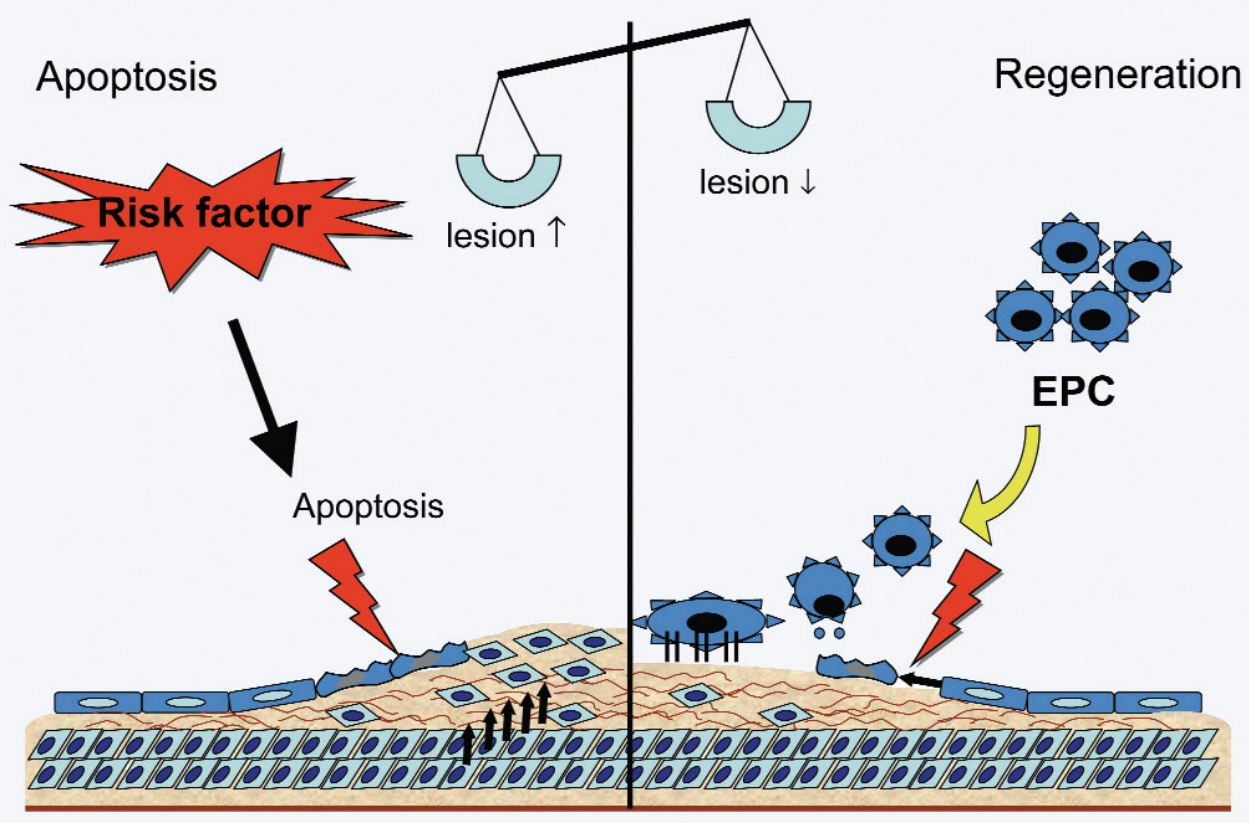


Fig. 5 Endothelial cell apoptosis and endothelial cell regeneration. Apoptotic endothelial cells are regenerated either by adjacent endothelial cells or - as shown recently - by circulating, bone marrow-derived endothelial progenitor cells. Under steady state conditions the integrity of the endothelium is assured due to effective endothelial cell repair. However, the system becomes imbalanced in conditions of enhanced endothelial cell apoptosis and impaired progenitor-mediated endothelial cell repair. The resulting disrupture of the endothelium which can not be effectively reconstituted, results in the development and progression of an atherosclerotic lesion.

other studies have demonstrated that we are meanwhile able to sufficiently mobilize stem and progenitor cells into peripheral blood. Besides erythropoietin, estrogen, statins, G-CSF, VEGF, and physical activity, various other agents have been shown to have beneficial effects on mobilization [27, 34, 37–40, 69–80]. However, the data concerning their role in improving progenitor cell function and homing remains limited.

Currently, the experimental proof that a mobilization therapy can effectively prevent the development of atherosclerotic lesion development is still missing and no data is available showing beneficial effects of the described substances in patients with risk factor-mediated progenitor cell dysfunction. Again, we have to rely on computer simulation models. Kravchenko *et al.* estimated the impact of progenitor cell therapy for atherosclerosis on cardiovascular mortality, life expectancy, and survival compared with the lifetime control of conventional risk factors [81]. In his model, a "virtual" progenitor cell therapy was applied at the age of 30 assuming a 10-year delay in atherosclerosis progression. Males on EPC therapy had the lowest projected cardiovascular mortality rate compared to patients with an "ideal" lifetime control of risk factors. EPC therapy showed an effect on life expectancy better than the complete elimination of cancer (in males, an additional 5.94 *vs.* 2.86 years). Together with the results of the EPCAD study we have strong evidence that a progenitor cell-based therapy may be a highly effective way to prevent atherosclerosis. The therapeutic goal must be the equalization of an imbalance between endothelial cell regeneration and apoptosis. In this context the use of a vascular repair index unifying the counteracting processes at

the vascular wall (damage *vs*. regeneration) may be helpful in order to choose therapeutic strategies with a maximized benefit for the patient. Future research will have to look in detail on the underlying molecular mechanisms of regeneration and risk factor-mediated dysfunction of regenerative cells in order to find more effective strategies for the prevention and therapy of atherosclerotic disease.

Acknowledgments

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