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 characteristic of cardiovascular risk factors. 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 • atherosclerosis
characterized 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
and up-take of acetylated low-density lipoprotein (LDL) cholesterol [4, 13]. However, it is unclear at present whether these cells resemble the circulating “naive” 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-
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 cotreatment 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 percutaneous coronary interventions. Clinical implications may be enor-
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-
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 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.

![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.](image-url)
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].

### Table: EPCAD Study Outcomes

| 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.
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 therapeutic 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 lifestyle 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
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

---

**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 disruption of the endothelium which can not be effectively reconstituted, results in the development and progression of an atherosclerotic lesion.
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 underly-
ing molecular mechanisms of regeneration and risk factor-mediated dysfunction of regenerative cells in order to find more effective strategies for the pre-
vention and therapy of atherosclerotic disease.

Acknowledgments

This work was supported by the European Vascular Genomics Network, a Network of Excellence granted by the European Commission (Contract No LSHM-CT-
2003-503254).

References

1. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardio-
diovascular events in patients with coronary artery disease. Circulation 2001; 104: 2673–8.
2. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000; 101: 1899–906.
3. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000; 101: 948–54.
4. Asahara T, Murohara T, Sullivan A, Silver M, van der ZR, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964–7.
5. Roberts N, Jahangiri M, Xu Q. Progenitor cells in vascular disease. J Cell Mol Med. 2005; 9: 583–91.
6. Rumpold H, Wolf D, Koeck R, Gunsilius E. Endothelial progenitor cells: a source for therapeutic vasculogenesis? J Cell Mol Med. 2004; 8: 509–18.
7. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res. 1999; 85: 221–8.
8. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. Circ Res. 2004; 95: 343–53.
9. Gill M, Dias S, Hattori K, Rivera ML, Hicklin D, Witte L, Girardi L, Yurt R, Himel H, Rafii S. Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells. Circ Res. 2001; 88: 167–74.
10. Handgretinger R, Gordon PR, Leimig T, Chen X, Buhring HJ, Niethammer D, Kuei S. Biology and plasticity of CD133+ hematopoietic stem cells. Ann N Y Acad Sci. 2003; 996: 141–51.
11. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vasculization and regeneration. Nat Med. 2003; 9: 702–12.
12. Friedrich EB, Walenta K, Scharlau J, Nickenig G, Werner N. CD34+/CD133+/VEGFR-2+ endothelial progenitor cell subpopulation with potent vasoregenerative capacities. Circ Res. 2006; 98: e20–5.
13. Vasa M, Fichtscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res. 2001; 89: E1–7.
14. Hill JM, Zalos G, HaGos JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial pro-
genitor cells, vascular function, and cardiovascular risk. N Engl J Med. 2003; 348: 593–600.
15. 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: 101–8.
16. Min TQ, Zhu CJ, Xiang WX, Hui ZJ, Peng SY. Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease. Cardiovasc Drugs Ther. 2004; 18: 203–9.
17. Loomans CJ, De Koning EJ, Staal FJ, Rookmaaker MB, Versyden C, De Boer HC, Verhaar MC, Braam B, Rabelink TJ, Van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes 2004; 53: 195–9.
18. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002; 106: 2781–6.
19. Krankel N, Adams V, Linke A, Gielen S, Erbs S, Lenk K, Schuler G, Hambrecht R. Hyperglycemia reduces survival and impairs function of circulating blood-derived progenitor cells. Arterioscler Thromb Vasc Biol. 2005; 25: 698–703.
20. Tamarat R, Silvestre JS, Ricousse-Roussanne S, Barateau V, Lecomte-Raclet L, Clergue M, Duriez M, Toebel G, Levy BI. 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: 457–66.
21. Wang CH, Ciliberti N, Li SH, Szmitko PE, Weisel RD, Fedak PW, Al Omran M, Cherng WJ, Li RK, Stanford WL, Verma S. Rosiglitazone facilitates angiogenic progenitor cell differentiation toward endothelial lineage: a new paradigm in glitazone pleiotropy. Circulation 2004; 109: 1392–400.
22. Bahlmann FH, De Groot K, Mueller O, Hertel B, Haller H, Fliser D. Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. *Hypertension* 2005; 45: 526–9.

23. 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: 273–80.

24. Wang X, Chen J, Tao Q, Zhu J, Shang Y. Effects of oxidized low-density lipoprotein on number and activity of circulating endothelial progenitor cells. *Drug Chem Toxicol.* 2004; 27: 243–55.

25. 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: 407–13.

26. Imanishi T, Hano T, Matsu O, Nishio I. Oxidized low-density lipoprotein inhibits vascular endothelial growth factor-induced endothelial progenitor cell differentiation. *Clin Exp Pharmacol Physiol.* 2003; 30: 665–70.

27. Dammeler S, Aicher A, Vasa M, Mıldner-Rühm C, Adler K, Tiemann M, Ruten H, Fichtlscherer S, Martin H, Zeiher AM. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest.* 2001; 108: 391–7.

28. Kondo T, Hayashi M, Takeshita K, Numaguchi Y, Kobayashi K, Iino S, Inden Y, Murohara T. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol.* 2004; 24: 1442–7.

29. 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: 881–9.

30. Chen JZ, Zhu JH, Wang XX, Zhu JH, Xie XD, Sun J, Shang YP, Guo XG, Dai HM, Hu SJ. Effects of homocysteine on number and activity of endothelial progenitor cells from peripheral blood. *J Mol Cell Cardiol.* 2004; 36: 233–9.

31. Suh W, Kim KL, Choi JH, Lee YS, Lee JY, Kim JM, Jang HS, Shin IS, Lee JS, Byun J, Jeon ES, Kim DK. C-reactive protein impairs angiogenic functions and decreases the secretion of arteriogenic chemo-cytokines in human endothelial progenitor cells. *Biochem Biophys Res Commun.* 2004; 321: 65–71.

32. Verma S, Kuliszewski MA, Li SH, Szmitko PE, Zucco L, Wang CH, Badiwala MV, Mickel DA, Weisel RD, Fedak PW, Stewart DJ, Kutryk MJ. 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: 2058–67.

33. Laufs U, Wassmann S, Czech T, Munzel T, Eisenhauer M, Bohm M, Nickenig G. Physical inactivity increases oxidative stress, endothelial dysfunction, and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2005; 25: 89–14.

34. Laufs U, Werner N, Link A, Endres M, Wassmann S, Jurgens K, Mische E, Bohm M, Nickenig G. Physical training increases endothelial progenitor cells, inhibits neointima formation, and enhances angiogenesis. *Circulation* 2004; 109: 220–6.

35. Sandri M, Adams V, Gielen S, Linke A, Lenk K, Kranckel N, Lenz D, Erbs S, Scheinert D, Mohr FW, Schuler G, Hambrecht R. 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: 3391–9.

36. Laufs U, Uhrhausen A, Werner N, Scharhag J, Heitz A, Kissner G, Bohm M, Kindermann W, Nickenig G. Running exercise of different duration and intensity: effect on endothelial progenitor cells in healthy subjects. *Eur J Cardiovasc Prev Rehabil.* 2005; 12: 407–14.

37. Strehal K, Werner N, Berweiler J, Link A, Dirnagl U, Priller J, Laufs K, Ghaeni L, Milopecic M, Bohm M, Nickenig G. Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. *Circulation* 2003; 107: 3059–65.

38. Kong D, Melo LG, Gneccchi M, Zhang L, Mostoslavsky G, Liew CC, Pratt RE, Dzau VJ. Cytokine-induced mobilization of circulating endothelial progenitor cells enhances repair of injured arteries. *Circulation* 2004; 110: 2039–46.

39. Werner N, Priller J, Laufs U, Endres M, Bohm M, Dirnagl U, Nickenig G. 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: 1567–72.

40. Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, Nishimura H, Losordo DW, Asahara T, Isner JM. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. *Circulation* 2002; 105: 3017–24.

41. George J, Herz I, Goldstein E, Abashidze S, Deutch V, Finkelstein A, Michowitz Y, Miller H, Keren G. Number and adhesive properties of circulating endothelial progenitor cells in patients with in-stent restenosis. *Arterioscler Thromb Vasc Biol.* 2003; 23: e57–60.

42. Aoki J, Serruys PW, van Beuskom H, Ong AT, McFadden EP, Sianos G, van der Giessen WJ, Regar E, de Feyter PJ, Davis HR, Rowland S, Kutryk MJ. Endothelial progenitor cell capture by stents coated with antibody against CD34: the HEALING-FIM (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth-First In Man) Registry. *J Am Coll Cardiol.* 2005; 45: 1574–9.

43. Shirota T, Yasui H, Matsuda T. Intraluminal tissue-engineered therapeutic stent using endothelial progenitor cell-inoculated hybrid tissue and in vitro performance. *Tissue Eng.* 2003; 9: 473–85.

44. Shirota T, Yasui H, Shimokawa H, Matsuda T. Fabrication of endothelial progenitor cell (EPC)-seeded intravascular stent devices and in vitro endothelialization on hybrid vascular tissue. *Biomaterials* 2003; 24: 2295–302.

45. Libby P. Inflammation in atherosclerosis. *Nature* 2002; 420: 868–74.

46. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, Wang T, Gregg D, Ramaswami P, Pippen AM,
Annex BH, Dong C, Taylor DA. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 2003; 108: 457–63.

Karra R, Vemullapalli S, Dong C, Herderick EE, Song X, Slosek K, Nevins JR, West M, Goldschmidt-Clermont PJ, Seo D. Molecular evidence for arterial repair in atherosclerosis. *Proc Natl Acad Sci USA*. 2005; 102: 16789–94.

Op dB, Musters M, Verrips T, Post JA, Braam B, van Riel N. Mathematical modeling of vascular endothelial layer maintenance: the role of endothelial cell division, progenitor cell homing, and telomere shortening. *Am J Physiol Heart Circ Physiol*. 2004; 287: H2651–8.

Stoll BR, Migliorini C, Kadambi A, Munn LL, Jain RK. A mathematical model of the contribution of endothelial progenitor cells to angiogenesis in tumors: implications for anti-angiogenic therapy. *Blood*. 2003.

Werner N, Kosiol S, Schieg T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med*. 2005; 353: 999–1007.

Moulton KS, Vakili K, Zurakowski D, Soliman M, Butterfield C, Sylvin E, Lo KM, Gillies S, Javaherian K, Folkman J. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. *Proc Natl Acad Sci USA*. 2003; 100: 4736–41.

Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynska H, Sharma SK, Badimon JJ, O’Connor WN. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. *Circulation* 2004; 110: 2032–8.

Hu Y, Davison F, Zhang Z, Xu Q. Endothelial replacement and angiogenesis in arteriosclerotic lesions of alo- grafts are contributed by circulating progenitor cells. *Circulation* 2003; 108: 3122–7.

Okazaki T, Ehbhar A, Asada M, Kanda A, Sasaki H, Yamaya M. Granulocyte colony-stimulating factor promotes tumor angiogenesis via increasing circulating endothelial progenitor cells and Gr1+CD11b+ cells in cancer animal models. *Int Immunol*. 2006; 18: 1–9.

Zhang H, Vakil V, Braunstein M, Smith EL, Maroney J, Chen L, Dai K, Berenson JR, Hussain MM, Kluempelberg U, Norin AJ, Akman HO, Ozelik T, Batuman OA. Circulating endothelial progenitor cells in multiple myeloma: implications and significance. *Blood*. 2005; 105: 3286–94.

Hilbe W, Dirnhofer S, Oberwasserlechner F, Schmid T, Gunsilius E, Hilbe G, Woll E, Kahler CM. CD133 positive endothelial progenitor cells contribute to the tumor vasculature in non-small cell lung cancer. *J Clin Pathol*. 2004; 57: 965–9.

Ribatti D. The involvement of endothelial progenitor cells in tumor angiogenesis. *J Cell Mol Med*. 2004; 8: 294–300.

Lee IG, Chae SL, Kim JC. Involvement of circulating endothelial progenitor cells and vasculogenic factors in the pathogenesis of diabetic retinopathy. *Eye*. 2005.

Moldovan NI. Angiogenesis, l’enfant terrible of vascular biology is coming of age. *J Cell Mol Med*. 2005; 9: 775–6.
Kleiner D, Templin C, Kotlarz D, Mueller M, Fuchs M, Hornig B, Haller H, Drexler H. 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: 1933–9.

73. Llevadot J, Murasawa S, Kureishi Y, Uchida S, Masuda H, Kawamoto A, Walsh K, Isner JM, Asahara T. HMG-CoA reductase inhibitor mobilizes bone marrow-derived endothelial progenitor cells. *J Clin Invest*. 2001; 108: 399–405.

74. Heissig B, Rafii S, Akiyama H, Ohki Y, Sato Y, Rafael T, Zhu Z, Hicklin DJ, Okumura K, Ogawa H, Werb Z, Hattori K. Low-dose irradiation promotes tissue revascularization through VEGF release from mast cells and MMP-9-mediated progenitor cell mobilization. *J Exp Med*. 2005; 202: 739–50.

75. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med*. 2003; 9: 1370–6.

76. Bahlmann FH, De Groot K, Spandau JM, Landry AL, Hertel B, Duckert T, Boehm SM, Menne J, Haller H, Fliser D. Erythropoietin regulates endothelial progenitor cells. *Blood* 2004; 103: 921–6.

77. Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher AM, Dimmeler S. Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. *Blood* 2003; 102: 1340–6.

78. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 2002; 109: 625–37.

79. Takamiya M, Okigaki M, Jin D, Takai S, Nozawa Y, Adachi Y, Urao N, Tateishi K, Nomura T, Zen K, Ashihara E, Miyazaki M, Tatsuki T, Takahashi T, Matsubara H. Granulocyte Colony-Stimulating Factor-mobilized circulating c-Kit+/Flk-1+ progenitor cells regenerate endothelium and inhibit neointimal hyperplasia after vascular injury. *Arterioscler Thromb Vasc Biol*. 2006.

80. Aicher A, Zeiher AM, Dimmeler S. Mobilizing endothelial progenitor cells. *Hypertension* 2005; 45: 321–5.

81. Kravchenko J, Goldschmidt-Clermont PJ, Powell T, Stallard E, Akushevich I, Cuffe MS, Manton KG. Endothelial progenitor cell therapy for atherosclerosis: the philosopher’s stone for an aging population? *Sci Aging Knowledge Environ*. 2005; 2005: e18.