forming vasculature and overall tissue regeneration opened in this regard unprecedented opportunities for the treatment of ischemic diseases, which thus far was bound to and limited by the classic paradigm of angiogenesis, marking the beginning of a new era in tissue regeneration previously not believed to be possible.

EPC transplantation in animal models

It was shown that therapeutic approaches utilizing culture-expanded EPCs could successfully promote neovascularization and regeneration of ischemic tissues, even when administered as sole therapy, that is, in the absence of other supportive pro-angiogenic growth factors. Such a supply-side version of therapeutic neovascularization in which the substrate (EPCs/ECs) rather than ligand (growth factor) comprises the therapeutic tool was first reported for the intravenous transplantation of human PB-derived cultured EPCs into immunodeficient mice with hindlimb ischemia (72). These experimental findings proved that exogenously administered EPCs could restore impaired neovascularization in a murine ischemic hindlimb model. A similar study in which human culture-expanded EPCs were transplanted in a nude rat myocardial ischemia model demonstrated that transplanted EPCs recruited to ischemic myocardium and were able to differentiate into ECs in sites of neovascularization. These findings were consistent with the observed preservation of left ventricular (LV) function and a reduction in infraction size (75, 76). Another study in which human cord blood-derived EPCs were transplanted in a nude rat hindlimb ischemia model also demonstrated similar findings with enhanced neovascularization in ischemic tissues (109).

Recently, several groups have explored the therapeutic potential of CD34+ cells as a possible EPC-enriched fraction. As described above, a clear distinction between HSCs and EPCs with the current available methodology in many cases is not possible, nonetheless is the use of the hematopoietic cell surface marker CD34, for isolation/enrichment of EPCs a widely used approach in the field of EPC biology. Shatteman *et al.* transplanted freshly isolated human CD34+ cells into diabetic nude mice with hindlimb ischemia, and showed significant blood flow recovery in ischemic limbs (131). Kocher *et al.* infused freshly isolated human CD34+ cells into a nude rat model of myocardial ischemia, and observed preservation of LV function and inhibition of cardiac apoptosis (80). Dose-dependent contribution of CD34+ cells to LV functional recovery and neovascularization in ischemic myocardium has also been demonstrated by Iwasaki *et al.* (70) (Tables 1 and 2).

EPC transplantation in clinical trials

Numerous clinical trials are now on going and trying to elucidate the therapeutic effects of EPCs seen in animal models on ischemic diseases, utilizing a broad range of cells that are all believed to consist of or contain, to a certain extent, EPCs and/or pro-vasculogenic/angiogenic cell populations (5, 40). Excellent in depth reviews summarizing the cells, conditions, as well as the therapeutic outcome, efficacy, and safety of the applied strategies are available (71, 94, 95) (Table 3).

One example for such a clinical study utilizing EPCs is our reported phase I/II clinical trial regarding the intramuscular transplantation of autologous and G-CSF-mobilized CD34+ cells in patients with intractable critical limb ischemia (77) (Fig. 7). The first-in-man trial has been conducted as a prospective, multicenter, single-blind, and dose-escalation study since 2002 in our institute. G-CSF was used to efficiently mobilize BM-EPCs into the PB, and the mobilized CD34+

TABLE 1. MOUSE ENDOTHELIAL PROGENITOR CELLS IN EXPERIMENTAL ANIMAL MODELS

Source	*Detection/isolation method*	*Cell surface marker(s)*	*Functional modulator*	*References*
BM	MACS	CD34−/CD14+	—	47
PB	FACS	CD34+/Flk-1+	Erythropoietin	48
BM	FACS	CD11b+/CD45+	—	165
BM	MACS	c-kit+/CD31+	MMP-9	54
PB	PB	c-kit+/CD31+	ApoA-I	31
BM	MACS	c-kit+	MMP-2	18
BM	FACS/MACS	c-kit+/Lin−	Nox2	157
PB	FACS	c-kit+/Tie-2+	Ischemic preconditioning	117
PB	FACS	c-kit+/Flk-1+/CD11b−	—	37
PB	FACS	c-kit+/Flk-1+/CD45+	Chronic hypoxia	99
BM	FACS/MACS	c-kit+/Sca-1+/Lin−	Lnk	73
BM	FACS/MACS	Sca-1+/Lin−	Lnk	87
BM	FACS	Sca-1+/c-kit+	Surgical injury	20
PB	FACS	Sca-1+/Flk-1+	Estrogen (45, 68, 69)/Enalapril (161)/HDL (31)	31, 45, 68, 69, 161
PB	FACS	Sca-1+/Flk-1+/c-kit+	Hepatocyte growth factor	66
BM	FACS	Sca-1+/CD11b+	BDNF	79
ES	FACS	Flk-1+/E-cadherin-	Presenilin-I	111
PB	FACS	CXCR4+/Flk-1+	Hypoxia/stromal cell-derived factor-1α	36
PB	FACS/MACS	CD45-/CD3−/CD31+/Tie-2+	NO	41
PB/BM	Culture	BS1-lectin/acLDL	Statin (21, 93)/glucose (61)/cholesterol (62)/ischemic preconditioning (59)	21, 59, 61, 62, 93

BDNF, brain-derived neurotrophic factor; BM, bone marrow; ES, embryonic stem; FACS, fluorescence-activated cell sorter; MACS, magnetically activated cell sorting; MMP, matrix metalloproteinase; NO, nitric oxide; PB, peripheral blood.

TABLE 2. HUMAN ENDOTHELIAL PROGENITOR CELLS IN PATIENTS AND EXPERIMENTAL ANIMAL MODELS

Source	*Detection/ isolation method*	*Cell surface markers*	*Background diseases*	*References*
PB	FACS	CD34+/KDR+	Cardiovascular disease (26, 27, 39, 52, 120, 129, 132, 140, 147) /diabetes (28, 160, 162) /cancer (23)/stroke (167) /others (49)	23, 26–28, 49, 52, 120, 121, 129, 132, 140, 147, 160, 162, 167
CB	FACS	CD34+/KDR+	NA	58
PB	FACS	CD34+/CD133+	Cardiovascular disease (122, 140, 154) /allergy (3, 9)/cancer (4, 168) /stroke (92)/others (113, 163)	3, 4, 9, 53, 84, 92, 113, 122, 140, 154, 163, 168
CB	FACS	CD34+/CD133+	NA	113
PB	FACS	CD34+/CD133+/KDR+	Cardiovascular disease (98, 100, 125, 158)/lung disease (29)/leukemia (57, 103, 128)/inflammation (43, 104, 138)/ischemic limb (166) /obesity (156)/training /hemodialysis (148)	29, 43, 57, 98, 100, 103, 104, 125, 128, 138, 146, 148, 156, 158, 166
PB	FACS/MACS	CD34+	Cardiovascular disease (70, 95, 114, 116, 139)/diabetes (133)/bone fracture (102, 106) /others (116, 143)	70, 95, 102, 106, 114, 116, 133, 139
CB	FACS/MACS	CD34+	Cardiovascular disease (116) /peripheral artery disease (109) /others (24, 30, 35, 50, 74, 96, 112, 141, 142)	24, 30, 35, 50, 74, 96, 109, 112, 116, 141, 142
PB	FACS	CD34+/CD31+	Cardiovascular disease (12) /stroke (167)	12, 167
PB	FACS	CD34+/CD133+/CD45+	Hypertension	115
PB	FACS	CD34+/Pselectin+	Stroke	167
PB	FACS	CD34+/ckit+/KDR+	AMI/stable angina pectoris	100
PB	FACS	CD133+/KDR+	Lung cancer (23)/aging (49, 153) /red wine intake (53)/cardiac rehabilitation (118)/coronary artery disease (122)	23, 49, 53, 118, 122, 153
CB	FACS	CD133+/KDR+	Pre-eclampsia (164)	58, 164
PB	FACS	CD133+	Ischemic flap	17
CB	MACS	CD133+	MI (134)	134, 135
PB	FACS	CD45+(dim+)/CD133+ /CD144+KDR+	Thermal injury	33
PB	FACS	CD45 (low+)/CD34+ /CD133+/KDR+	Smoking	82
PB	FACS	CD34-/CD133+/KDR+	NA	34
PB	FACS	CD3-/CD34+/KDR+	Coronary artery disease	1

CB, cord blood; NA, not available.

cells were isolated as an EPC-enriched fraction. In all subjects, primary endpoint of efficacy score at week 12 was positive, indicating improvement of lower limb ischemia after cell therapy. In addition, both subjective and objective parameters of lower limb ischemia such as toe brachial pressure index, transcutaneous partial oxygen pressure, total walking distance, pain-free walking distance, Wang-Baker's pain rating scale, and ulcer size improved significantly after the transplantation of CD34+ cells (Fig. 7). Because this was not a controlled, randomized study, the possibility of a placebo effect after CD34+ cell transplantation needs to be evaluated in a large-scale trial in the future. As for the evaluation of safety issues, neither death nor life-threatening adverse events were observed in this study, and no severe adverse events except for transient and expected mild to moderate ones could be observed as a result of the performed cell therapeutic approach. These outcomes suggest the safety and feasibility of this cell-based therapy in patients with critical limb ischemia.

Problems in EPC transplantation

Our animal studies as well as the results of other groups suggest that heterologous EPC transplantation requires systemic injections of $0.5 \sim 2.0 \times 10^4$ human EPCs/g body weight of the recipient animal to achieve a satisfactory improvement of hindlimb ischemia (6, 55, 70, 72, 75). In general, cultured EPCs obtained from healthy human volunteers yield 5.0×10^6 cells per 100 ml of PB on day 7. Based on these data in human, a blood volume of as much as 12 l will be necessary to obtain a sufficient number of EPCs for the treatment of patients with critical hindlimb ischemia. Therefore, the background factors

TABLE 3. CLINICAL TRIALS FOR ISCHEMIC DISEASES WITH ENDOTHELIAL PROGENITOR CELLS

Trial name/ author	*Disease type*	*Number of patients (T/C)*	*EPC type*	*Study design*	*Outcome*	*References*
TOPCARE-AMI	AMI	30/29	PB-/BM-derived Cultured EPCs	RT	Effective	130
Bartunek *et al.*	AMI	19/16	CD133	RT	Effective	13
Li *et al.*	AMI	35/35	Gm-PB-CD34	Cohort	Effective	90
Tatsumi *et al.*	AMI	36/18	PB-mononuclear cell	Cohort	Effective	152
Dobert *et al.*	AMI	11/15	PB-/BM-derived Cultured EPCs	Cohort	Effective	22
Stamm *et al.*	RMI	46/9	CD133	NRT	Effective	144, 145
Ahmadi *et al.*	RMI	18/9	CD133	NRT	Effective	2
Balogh *et al.*	RMI	8/18	CD34	NRT	Inconclusive	11
Erbs *et al*	OMI	13/13	PB-derived Cultured EPCs	RT	Effective	25
Assmus *et al.*	OMI	24/23	PB-derived Cultured EPCs	RCT	Ineffective	10
Boyle *et al.*	OMI	5/0	Gm-PB-CD34	NRT	NA	16
Losordo *et al.*	AP	18/6	Gm-PB-CD34	RT	Safe and feasible	95
Lara-Hernandez *et al.*	CLI	28/0	Gm-PB-CD34	Cohort	Effective	88
EPOCH-CLI	CLI	17/0	Gm-PB-CD34	Cohort	Effective	77
Kuroda *et al.*	NUF	4/0	Gm-PB-CD34	Cohort	Effective	Ongoing in Kobe, Japan

AMI, acute myocardial infarction; AP, angina pectoris; CLI, critical limb ischemia; EPC, endothelial progenitor cell; Gm, G-CSF (granulocyte colony-stimulating factor) mobilized; NA, not available; NRT, nonrandomized trial; NUF, nonunion fracture; OMI, old myocardial infarction; RMI, recent myocardial infarction; RT, randomized trial; T/C, treatment/control.

in clinical patients such as aging (49), diabetes (61, 159), hypercholesterolemia (159), hypertension (63, 159), and smoking (82, 105) that may reduce the number and biological activity of circulating/BM-EPCs represent possible major limitations for the success of primary EPC transplantations. In reality, most of the patients who are going to undergo EPC therapy for ischemic diseases have background diseases as described above. Considering autologous EPC therapy, certain technical improvements that may help to overcome the shortcomings of EPCs should include (i) local delivery of EPCs, (ii) endogenous EPC mobilization, that is, cytokine/growth factor supplementation to promote BM-derived EPC mobilization (8, 149), (iii) enrichment procedures, that is, leukapheresis or BM aspiration, (iv) enhancement of EPC functions by gene transduction, or (v) culture expansion of EPCs from self-renewable primitive stem/progenitor cells isolated from BM or other sources. Unless the quality and quantity of autologous EPCs can be increased by the introduction of such technical improvements as mentioned above, allogenic EPCs derived from UCB or culture-expanded/generated EPCs from human ES/iPS cells (89, 109) may serve as alternative sources for the supply of therapeutic active EPCs.

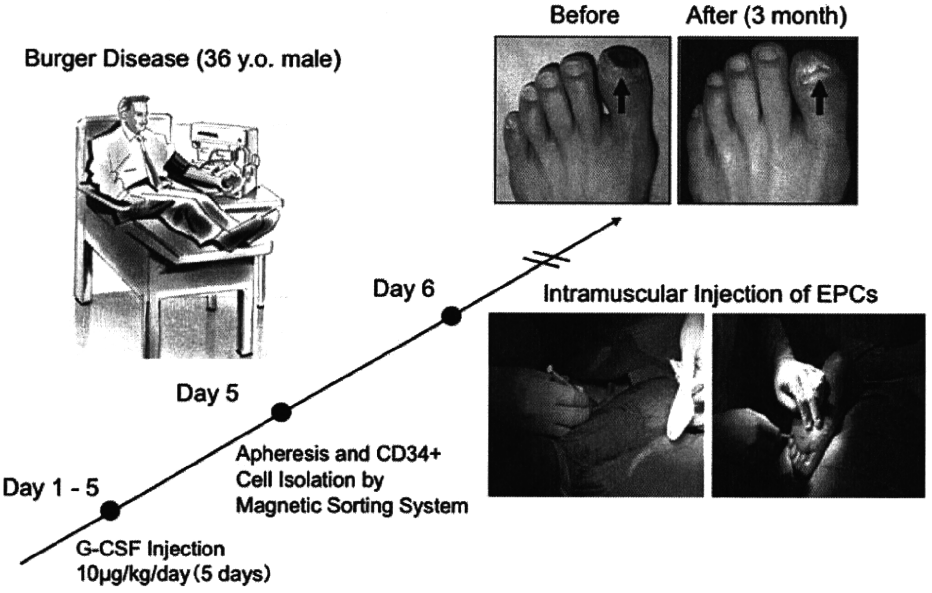

FIG. 7. Representative case of autologous CD34+ cell transplantation therapy for critical limb ischemia in Burger disease. A 36-year-old male patient who had toe necrosis due to microcirculation failure received CD34+ cell injection at 40 sites in ischemic limb under lumbar anesthesia, and the necrosis was significantly improved with blood flow recovery with reduced skin ulcer size 3 months after the treatment. The improvement could be maintained for more than 1 year without recurrence and any adverse side effects. *Arrow* indicates a part of necrosis before and after the treatment.

Future strategies for EPC-based therapeutic angiogenesis

Strategies that will recover potential EPC dysfunction and improve the bioactivity of these cells for the successful treatment of ischemic disorders should be considered, especially, in light of the current findings, implicating that EPC function and mobilization may be impaired in certain diseases. One such strategy, the genetic modification and enhancement of EPCs with, for example, the targeted overexpression of pro-angiogenic growth factors in these cells, may enhance the angiogenic response and reactivate the bioactivity and/or extend the life span of EPCs.

In terms of increasing the quality of EPCs, we have shown that gene-modified EPCs could rescue impaired neovascularization in an animal model of limb ischemia (67). Transplantation of heterologous EPCs transduced with adenovirus encoding human VEGF165 improved neovascularization and blood flow recovery, reducing the limb necrosis and auto-amputation rate in comparison with controls. The dose of EPCs needed to achieve limb salvage in these *in vivo* experiments was 30 times less than that required in the previous experiments involving unmodified EPCs (72). Other investigators have also demonstrated the therapeutic efficacy of genetically engineered EPCs with a variety of targeted genes such as adrenomedullin (110), *eNOS* (83), tissue plasminogen activator (42), and integrin-like kinase (19) in animal models. Thus, genetic modification might overcome the potential problems associated with less potent patient EPCs and increase the therapeutic efficacy of such approaches, possibly, leading to the widespread use of these so-called second generation EPC therapies. Combination of EPC cell-based therapy with gene (*i.e., VEGF*) therapy (78) or the combined use of pro-angiogenic, vascular stabilizing factors, that is, Ang-1 and vasohibin-2, may also improve and overcome some of the current limitations experienced in the field. In addition, we have also recently succeeded in expanding freshly isolated G-CSF-mobilized PB-derived human CD34+ cells up to 20–50 times of their original cell number with growth factor/cytokine cocktail-supplemented medium under serum-free conditions (Fig. 8). Further, these culture-expanded hCD34+ cells exhibited higher therapeutic efficacy *in vivo* showing also increased pro-angiogenic cytokine expressions *in vitro* compared to freshly isolated hCD34+ cells (unpublished data). This strategy might compensate the current disadvantages of applying dysfunctional EPCs for autologous cell transplantation therapy in ischemic diseases by increasing the quantity and quality of the applied EPCs.

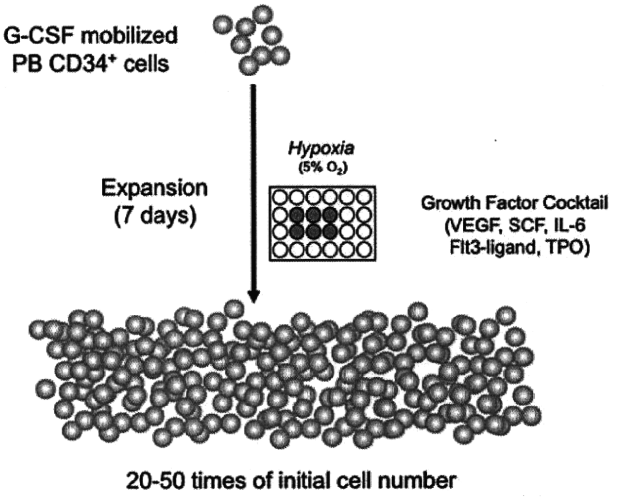

FIG. 8. ***Ex vivo*** **culture expansion of human CD34+ cells.** G-CSF-mobilized PB-mononuclear cells are cultured in a serum-free defined medium supplemented with a cytokine/growth factor cocktail (VEGF, vascular endothelial growth factor; SCF, stem cell factor; IL-6, interleukin-6; Flt3, a ligand; TPO, thrombopoietin) under 5% O_2 condition for 7 days. The number of expanded CD34+ cells can reach up to 20–50 times of the original cell number.

Summary

Accumulating evidence suggests that BM-derived EPCs have the potential to promote postnatal vasculogenesis in adults, thus opening the way for possible clinical applications and the targeted cellular therapy of cardio-vascular diseases. For a successful therapeutic EPC-based approach, the isolation and preparation of an optimal quality/quantity of EPCs is essential, making the resolution of certain still unresolved issues in the field a pressing prerequirement, such as (i) the development of better and more efficient EPC purification and expansion methods, (ii) the improvement of administration and cellular application techniques, and (iii) the recovery of the disease-based dysfunction and/or senescence of patient-derived EPCs.

All these above-mentioned aspects and awaited breakthroughs are undeniably also linked to and hampered by the lack of a clear and unambiguous definition of EPCs and the shear multitude of proposed EPC phenotypes, some of which we have tried to introduce and summarize earlier. An unified and generally accepted definition of EPCs, currently comprising a huge variety of cells used for animal studies or clinical applications, allowing a detailed and meaningful molecular and/or functional characterization of these cells, and permitting subsequent proper interpretation of the observed preclinical and/or clinical phenotypes, is unfortunately still missing. Surprisingly, the huge variety of applied EPCs ranging from HSC-related CD34/CD133-positive cells to utterly heterogeneous cell populations like BM-MNCs seems to be still acceptable for the majority of pragmatic clinical investigators desperately searching for any working tools in their fight against cardiovascular diseases. The use of easily accessible cell populations like BM/PB-derived CD34+ cells will likely continue into the near future, especially with technical improvements regarding the isolation and application of these cells as well as the introduction of novel approaches of pretreatment and modification possibly further increasing the angiogenic properties of these cells. Other easily accessible cell types like mesenchymal stem cells or novel subpopulations of MNCs like CD31+ cells as well as ES/IP-derived populations of patient-specific endothelial (progenitor) cells may also enter the arena of vascular therapy in the near future, especially after overcoming current technical limitations of proper generation and propagation of such cell populations.

The rather sobering results of many clinical trials assessing the efficacy and safety of EPCs for the treatment of vascular diseases should thus be taken as an indication that many of the still remaining basic questions regarding EPCs, like the ultimate clarification and characterization of all phenotypical

manifestations of EPCs, should be taken seriously and addressed before real breakthroughs in the use of this promising tool can be anticipated.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Adams V, Lenk K, Linke A, Lenz D, Erbs S, Sandri M, Tarnok A, Gielen S, Emmrich F, Schuler G, and Hambrecht R. Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. *Arterioscler Thromb Vasc Biol* 24: 684–690, 2004.
2. Ahmadi H, Baharvand H, Ashtiani SK, Soleimani M, Sadeghian H, Ardekani JM, Mehrjerdi NZ, Kouhkan A, Namiri M, Madani-Civi M, Fattahi F, Shahverdi A, and Dizaji AV. Safety analysis and improved cardiac function following local autologous transplantation of CD133(+) enriched bone marrow cells after myocardial infarction. *Curr Neurovasc Res* 4: 153–160, 2007.
3. Allanore Y, Batteux F, Avouac J, Assous N, Weill B, and Kahan A. Levels of circulating endothelial progenitor cells in systemic sclerosis. *Clin Exp Rheumatol* 25: 60–66, 2007.
4. Arbab AS, Pandit SD, Anderson SA, Yocum GT, Bur M, Frenkel V, Khuu HM, Read EJ, and Frank JA. Magnetic resonance imaging and confocal microscopy studies of magnetically labeled endothelial progenitor cells trafficking to sites of tumor angiogenesis. *Stem Cells* 24: 671–678, 2006.
5. Asahara T and Kawamoto A. Endothelial progenitor cells for postnatal vasculogenesis. *Am J Physiol Cell Physiol* 287: C572–C579, 2004.
6. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, and Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 85: 221–228, 1999.
7. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, and Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964–967, 1997.
8. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, and Isner JM. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J* 18: 3964–3972, 1999.
9. Asosingh K, Swaidani S, Aronica M, and Erzurum SC. Th1- and Th2-dependent endothelial progenitor cell recruitment and angiogenic switch in asthma. *J Immunol* 178: 6482–6494, 2007.
10. Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, Teupe C, Pistorius K, Martin H, Abolmaali ND, Tonn T, Dimmeler S, and Zeiher AM. Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 355: 1222–1232, 2006.
11. Balogh L, Czuriga I, Hunyadi J, Galuska L, Kristof E, and Edes I. [Effects of autologous bone marrow derived CD34+ stem cells on the left ventricular function following myocardial infarction]. *Orv Hetil* 148: 243–249, 2007.
12. Banerjee S, Brilakis E, Zhang S, Roesle M, Lindsey J, Philips B, Blewett CG, and Terada LS. Endothelial progenitor cell mobilization after percutaneous coronary intervention. *Atherosclerosis* 189: 70–75, 2006.
13. Bartunek J, Vanderheyden M, Vandekerckhove B, Mansour S, De Bruyne B, De Bondt P, Van Haute I, Lootens N, Heyndrickx G, and Wijns W. Intracoronary injection of CD133-positive enriched bone marrow progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. *Circulation* 112: I178–I183, 2005.
14. Bauer SM, Goldstein LJ, Bauer RJ, Chen H, Putt M, and Velazquez OC. The bone marrow-derived endothelial progenitor cell response is impaired in delayed wound healing from ischemia. *J Vasc Surg* 43: 134–141, 2006.
15. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, and Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114: 763–776, 2003.
16. Boyle AJ, Whitbourn R, Schlicht S, Krum H, Kocher A, Nandurkar H, Bergmann S, Daniell M, O'Day J, Skerrett D, Haylock D, Gilbert RE, and Itescu S. Intra-coronary high-dose CD34+ stem cells in patients with chronic ischemic heart disease: a 12-month follow-up. *Int J Cardiol* 109: 21–27, 2006.
17. Chang EI, Loh SA, Ceradini DJ, Lin SE, Bastidas N, Aarabi S, Chan DA, Freedman ML, Giaccia AJ, and Gurtner GC. Age decreases endothelial progenitor cell recruitment through decreases in hypoxia-inducible factor 1alpha stabilization during ischemia. *Circulation* 116: 2818–2829, 2007.
18. Cheng XW, Kuzuya M, Nakamura K, Maeda K, Tsuzuki M, Kim W, Sasaki T, Liu Z, Inoue N, Kondo T, Jin H, Numaguchi Y, Okumura K, Yokota M, and Iguchi A, Murohara T. Mechanisms underlying the impairment of ischemia-induced neovascularization in matrix metalloproteinase 2-deficient mice. *Circ Res* 100: 904–913, 2007.
19. Cho HJ, Youn SW, Cheon SI, Kim TY, Hur J, Zhang SY, Lee SP, Park KW, Lee MM, Choi YS, Park YB, and Kim HS. Regulation of endothelial cell and endothelial progenitor cell survival and vasculogenesis by integrin-linked kinase. *Arterioscler Thromb Vasc Biol* 25: 1154–1160, 2005.
20. Condon ET, Wang JH, and Redmond HP. Surgical injury induces the mobilization of endothelial progenitor cells. *Surgery* 135: 657–661, 2004.
21. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rutten H, Fichtlscherer S, Martin H, and Zeiher AM. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 108: 391–397, 2001.
22. Dobert N, Britten M, Assmus B, Berner U, Menzel C, Lehmann R, Hamscho N, Schachinger V, Dimmeler S, Zeiher AM, and Grunwald F. Transplantation of progenitor cells after reperfused acute myocardial infarction: evaluation of perfusion and myocardial viability with FDG-PET and thallium SPECT. *Eur J Nucl Med Mol Imaging* 31: 1146–1151, 2004.
23. Dome B, Timar J, Dobos J, Meszaros L, Raso E, Paku S, Kenessey I, Ostoros G, Magyar M, Ladanyi A, Bogos K, and Tovari J. Identification and clinical significance of circulating endothelial progenitor cells in human non-small cell lung cancer. *Cancer Res* 66: 7341–7347, 2006.
24. Eggermann J, Kliche S, Jarmy G, Hoffmann K, Mayr-Beyrle U, Debatin KM, Waltenberger J, and Beltinger C. Endothelial progenitor cell culture and differentiation *in vitro*: a methodological comparison using human umbilical cord blood. *Cardiovasc Res* 58: 478–486, 2003.

25. Erbs S, Linke A, Adams V, Lenk K, Thiele H, Diederich KW, Emmrich F, Kluge R, Kendziorra K, Sabri O, Schuler G, and Hambrecht R. Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. *Circ Res* 97: 756–762, 2005.
26. Fadini GP, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A, and de Kreutzenberg SV. Peripheral blood CD34+ KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population. *Stroke* 37: 2277–2282, 2006.
27. Fadini GP, de Kreutzenberg S, Albiero M, Coracina A, Pagnin E, Baesso I, Cignarella A, Bolego C, Plebani M, Nardelli GB, Sartore S, Agostini C, and Avogaro A. Gender differences in endothelial progenitor cells and cardiovascular risk profile: the role of female estrogens. *Arterioscler Thromb Vasc Biol* 28: 997–1004, 2008.
28. Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, Grego F, Menegolo M, de Kreutzenberg SV, Tiengo A, Agostini C, and Avogaro A. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. *J Am Coll Cardiol* 45: 1449–1457, 2005.
29. Fadini GP, Schiavon M, Cantini M, Baesso I, Facco M, Miorin M, Tassinato M, de Kreutzenberg SV, Avogaro A, and Agostini C. Circulating progenitor cells are reduced in patients with severe lung disease. *Stem Cells* 24: 1806–1813, 2006.
30. Fan CL, Li Y, Gao PJ, Liu JJ, Zhang XJ, and Zhu DL. Differentiation of endothelial progenitor cells from human umbilical cord blood CD 34+ cells *in vitro*. *Acta Pharmacol Sin* 24: 212–218, 2003.
31. Feng Y, Jacobs F, Van Craeyveld E, Brunaud C, Snoeys J, Tjwa M, Van Linthout S, and De Geest B. Human ApoA-I transfer attenuates transplant arteriosclerosis via enhanced incorporation of bone marrow-derived endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 28: 278–283, 2008.
32. Folkman J and Shing Y. Angiogenesis. *J Biol Chem* 267: 10931–10934, 1992.
33. Fox A, Smythe J, Fisher N, Tyler MP, McGrouther DA, Watt SM, and Harris AL. Mobilization of endothelial progenitor cells into the circulation in burned patients. *Br J Surg* 95: 244–251, 2008.
34. Friedrich EB, Walenta K, Scharlau J, Nickenig G, and Werner N. CD34-/CD133+/VEGFR-2+ endothelial progenitor cell subpopulation with potent vasoregenerative capacities. *Circ Res* 98: e20–e25, 2006.
35. Furuhata S, Ando K, Oki M, Aoki K, Ohnishi S, Aoyagi K, Sasaki H, Sakamoto H, Yoshida T, and Ohnami S. Gene expression profiles of endothelial progenitor cells by oligonucleotide microarray analysis. *Mol Cell Biochem* 298: 125–138, 2007.
36. Gallagher KA, Liu ZJ, Xiao M, Chen H, Goldstein LJ, Buerk DG, Nedeau A, Thom SR, and Velazquez OC. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. *J Clin Invest* 117: 1249–1259, 2007.
37. Gao D, Nolan DJ, Mellick AS, Bambino K, McDonnell K, and Mittal V. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. *Science* 319: 195–198, 2008.
38. Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N, Kluge K, Schafer B, Hossfeld DK, and Fiedler W. *In vitro* differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 95: 3106–3112, 2000.
39. Giannotti G, Doerries C, Mocharla PS, Mueller MF, Bahlmann FH, Horvath T, Jiang H, Sorrentino SA, Steenken N, Manes C, Marzilli M, Rudolph KL, Luscher TF, Drexler H, and Landmesser U. Impaired endothelial repair capacity of early endothelial progenitor cells in prehypertension: relation to endothelial dysfunction. *Hypertension* 55: 1389–1397, 2010.
40. Goldberg JL and Laughlin MJ. UC blood hematopoietic stem cells and therapeutic angiogenesis. *Cytotherapy* 9: 4–13, 2007.
41. Goldstein LJ, Gallagher KA, Bauer SM, Bauer RJ, Baireddy V, Liu ZJ, Buerk DG, Thom SR, and Velazquez OC. Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. *Stem Cells* 24: 2309–2318, 2006.
42. Griese DP, Achatz S, Batzlsperger CA, Strauch UG, Grumbeck B, Weil J, and Riegger GA. Vascular gene delivery of anticoagulants by transplantation of retrovirally-transduced endothelial progenitor cells. *Cardiovasc Res* 58: 469–477, 2003.
43. Grisar J, Aletaha D, Steiner CW, Kapral T, Steiner S, Seidinger D, Weigel G, Schwarzinger I, Wolozcszuk W, Steiner G, and Smolen JS. Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. *Circulation* 111: 204–211, 2005.
44. Gulati R, Jevremovic D, Peterson TE, Chatterjee S, Shah V, Vile RG, and Simari RD. Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. *Circ Res* 93: 1023–1025, 2003.
45. Hamada H, Kim MK, Iwakura A, Ii M, Thorne T, Qin G, Asai J, Tsutsumi Y, Sekiguchi H, Silver M, Wecker A, Bord E, Zhu Y, Kishore R, and Losordo DW. Estrogen receptors alpha and beta mediate contribution of bone marrow-derived endothelial progenitor cells to functional recovery after myocardial infarction. *Circulation* 114: 2261–2270, 2006.
46. Handgretinger R, Gordon PR, Leimig T, Chen X, Buhring HJ, Niethammer D, and Kuci S. Biology and plasticity of CD133+ hematopoietic stem cells. *Ann N Y Acad Sci* 996: 141–151, 2003.
47. Harraz M, Jiao C, Hanlon HD, Hartley RS, and Schatteman GC. CD34- blood-derived human endothelial cell progenitors. *Stem Cells* 19: 304–312, 2001.
48. Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher AM, and Dimmeler S. Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. *Blood* 102: 1340–1346, 2003.
49. Heiss C, Keymel S, Niesler U, Ziemann J, Kelm M, and Kalka C. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 45: 1441–1448, 2005.
50. Hildbrand P, Cirulli V, Prinsen RC, Smith KA, Torbett BE, Salomon DR, and Crisa L. The role of angiopoietins in the development of endothelial cells from cord blood CD34+ progenitors. *Blood* 104: 2010–2019, 2004.
51. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, and Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 348: 593–600, 2003.
52. Hristov M, Fach C, Becker C, Heussen N, Liehn EA, Blindt R, Hanrath P, and Weber C. Reduced numbers of circulating endothelial progenitor cells in patients with coronary artery disease associated with long-term statin treatment. *Atherosclerosis* 192: 413–420, 2007.

53. Huang PH, Chen YH, Tsai HY, Chen JS, Wu TC, Lin FY, Sata M, Chen JW, and Lin SJ. Intake of red wine increases the number and functional capacity of circulating endothelial progenitor cells by enhancing nitric oxide bioavailability. *Arterioscler Thromb Vasc Biol* 30: 869–877, 2010.
54. Huang PH, Chen YH, Wang CH, Chen JS, Tsai HY, Lin FY, Lo WY, Wu TC, Sata M, Chen JW, and Lin SJ. Matrix metalloproteinase-9 is essential for ischemia-induced neovascularization by modulating bone marrow-derived endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 29: 1179–1184, 2009.
55. Hung HS, Shyu WC, Tsai CH, Hsu SH, and Lin SZ. Transplantation of endothelial progenitor cells as therapeutics for cardiovascular diseases. *Cell Transplant* 18: 1003–1012, 2009.
56. Hur J, Yang HM, Yoon CH, Lee CS, Park KW, Kim JH, Kim TY, Kim JY, Kang HJ, Chae IH, Oh BH, Park YB, and Kim HS. Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies. *Circulation* 116: 1671–1682, 2007.
57. Igreja C, Courinha M, Cachaco AS, Pereira T, Cabecadas J, da Silva MG, and Dias S. Characterization and clinical relevance of circulating and biopsy-derived endothelial progenitor cells in lymphoma patients. *Haematologica* 92: 469–477, 2007.
58. Igreja C, Fragoso R, Caiado F, Clode N, Henriques A, Camargo L, Reis EM, and Dias S. Detailed molecular characterization of cord blood-derived endothelial progenitors. *Exp Hematol* 36: 193–203, 2008.
59. Ii M, Nishimura H, Iwakura A, Wecker A, Eaton E, Asahara T, and Losordo DW. Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via "imported" nitric oxide synthase activity. *Circulation* 111: 1114–1120, 2005.
60. Ii M, Nishimura H, Sekiguchi H, Kamei N, Yokoyama A, Horii M, and Asahara T. Concurrent vasculogenesis and neurogenesis from adult neural stem cells. *Circ Res* 105: 860–868, 2009.
61. Ii M, Takenaka H, Asai J, Ibusuki K, Mizukami Y, Maruyama K, Yoon YS, Wecker A, Luedemann C, Eaton E, Silver M, Thorne T, and Losordo DW. Endothelial progenitor thrombospondin-1 mediates diabetes-induced delay in reendothelialization following arterial injury. *Circ Res* 98: 697–704, 2006.
62. Ii M, Takeshita K, Ibusuki K, Luedemann C, Wecker A, Eaton E, Thorne T, Asahara T, Liao JK, and Losordo DW. Notch signaling regulates endothelial progenitor cell activity during recovery from arterial injury in hypercholesterolemic mice. *Circulation* 121: 1104–1112, 2010.
63. Imanishi T, Moriwaki C, Hano T, and Nishio I. Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. *J Hypertens* 23: 1831–1837, 2005.
64. Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, and Yoder MC. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood* 105: 2783–2786, 2005.
65. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, and Yoder MC. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 104: 2752–2760, 2004.
66. Ishizawa K, Kubo H, Yamada M, Kobayashi S, Suzuki T, Mizuno S, Nakamura T, and Sasaki H. Hepatocyte growth factor induces angiogenesis in injured lungs through mobilizing endothelial progenitor cells. *Biochem Biophys Res Commun* 324: 276–280, 2004.
67. Iwaguro H, Yamaguchi J, Kalka C, Murasawa S, Masuda H, Hayashi S, Silver M, Li T, Isner JM, and Asahara T. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation* 105: 732–738, 2002.
68. Iwakura A, Luedemann C, Shastry S, Hanley A, Kearney M, Aikawa R, Isner JM, Asahara T, and Losordo DW. Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. *Circulation* 108: 3115–3121, 2003.
69. Iwakura A, Shastry S, Luedemann C, Hamada H, Kawamoto A, Kishore R, Zhu Y, Qin G, Silver M, Thorne T, Eaton L, Masuda H, Asahara T, and Losordo DW. Estradiol enhances recovery after myocardial infarction by augmenting incorporation of bone marrow-derived endothelial progenitor cells into sites of ischemia-induced neovascularization via endothelial nitric oxide synthase-mediated activation of matrix metalloproteinase-9. *Circulation* 113: 1605–1614, 2006.
70. Iwasaki H, Kawamoto A, Ishikawa M, Oyamada A, Nakamori S, Nishimura H, Sadamoto K, Horii M, Matsumoto T, Murasawa S, Shibata T, Suehiro S, and Asahara T. Dose-dependent contribution of CD34-positive cell transplantation to concurrent vasculogenesis and cardiomyogenesis for functional regenerative recovery after myocardial infarction. *Circulation* 113: 1311–1325, 2006.
71. Jujo K, Ii M, and Losordo DW. Endothelial progenitor cells in neovascularization of infarcted myocardium. *J Mol Cell Cardiol* 45: 530–544, 2008.
72. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, and Asahara T. Transplantation of *ex vivo* expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A* 97: 3422–3427, 2000.
73. Kamei N, Kwon SM, Alev C, Ishikawa M, Yokoyama A, Nakanishi K, Yamada K, Horii M, Nishimura H, Takaki S, Kawamoto A, Ii M, Akimaru H, Tanaka N, Nishikawa S, Ochi M, and Asahara T. Lnk deletion reinforces the function of bone marrow progenitors in promoting neovascularization and astrogliosis following spinal cord injury. *Stem Cells* 28: 365–375, 2010.
74. Kang HJ, Kim SC, Kim YJ, Kim CW, Kim JG, Ahn HS, Park SI, Jung MH, Choi BC, and Kimm K. Short-term phytohaemagglutinin-activated mononuclear cells induce endothelial progenitor cells from cord blood CD34+ cells. *Br J Haematol* 113: 962–969, 2001.
75. Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM, and Asahara T. Therapeutic potential of *ex vivo* expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 103: 634–637, 2001.
76. Kawamoto A, Iwasaki H, Kusano K, Murayama T, Oyamada A, Silver M, Hulbert C, Gavin M, Hanley A, Ma H, Kearney M, Zak V, Asahara T, and Losordo DW. CD34-positive cells exhibit increased potency and safety for therapeutic neovascularization after myocardial infarction compared with total mononuclear cells. *Circulation* 114: 2163–2169, 2006.
77. Kawamoto A, Katayama M, Handa N, Kinoshita M, Takano H, Horii M, Sadamoto K, Yokoyama A, Yamanaka T,

Onodera R, Kuroda A, Baba R, Kaneko Y, Tsukie T, Kurimoto Y, Okada Y, Kihara Y, Morioka S, Fukushima M, and Asahara T. Intramuscular transplantation of G-CSF-mobilized CD34(+) cells in patients with critical limb ischemia: a phase I/IIa, multicenter, single-blinded, dose-escalation clinical trial. *Stem Cells* 27: 2857–2864, 2009.

78. Kawamoto A, Murayama T, Kusano K, Ii M, Tkebuchava T, Shintani S, Iwakura A, Johnson I, von Samson P, Hanley A, Gavin M, Curry C, Silver M, Ma H, Kearney M, and Losordo DW. Synergistic effect of bone marrow mobilization and vascular endothelial growth factor-2 gene therapy in myocardial ischemia. *Circulation* 110: 1398–1405, 2004.
79. Kermani P, Rafii D, Jin DK, Whitlock P, Schaffer W, Chiang A, Vincent L, Friedrich M, Shido K, Hackett NR, Crystal RG, Rafii S, and Hempstead BL. Neurotrophins promote revascularization by local recruitment of TrkB+ endothelial cells and systemic mobilization of hematopoietic progenitors. *J Clin Invest* 115: 653–663, 2005.
80. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, and Itescu S. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 7: 430–436, 2001.
81. Kolbe M, Dohle E, Katerla D, Kirkpatrick CJ, and Fuchs S. Enrichment of outgrowth endothelial cells in high and low colony-forming cultures from peripheral blood progenitors. *Tissue Eng Part C Methods* 16: 877–886, 2010.
82. Kondo T, Hayashi M, Takeshita K, Numaguchi Y, Kobayashi K, Iino S, Inden Y, and Murohara T. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol* 24: 1442–1447, 2004.
83. Kong D, Melo LG, Mangi AA, Zhang L, Lopez-Ilasaca M, Perrella MA, Liew CC, Pratt RE, and Dzau VJ. Enhanced inhibition of neointimal hyperplasia by genetically engineered endothelial progenitor cells. *Circulation* 109: 1769–1775, 2004.
84. Korbling M, Reuben JM, Gao H, Lee BN, Harris DM, Cogdell D, Giralt SA, Khouri IF, Saliba RM, Champlin RE, Zhang W, and Estrov Z. Recombinant human granulocyte-colony-stimulating factor-mobilized and apheresis-collected endothelial progenitor cells: a novel blood cell component for therapeutic vasculogenesis. *Transfusion* 46: 1795–1802, 2006.
85. Kovacic JC and Boehm M. Resident vascular progenitor cells: an emerging role for non-terminally differentiated vessel-resident cells in vascular biology. *Stem Cell Res* 2: 2–15, 2009.
86. Kwon SM, Eguchi M, Wada M, Iwami Y, Hozumi K, Iwaguro H, Masuda H, Kawamoto A, and Asahara T. Specific Jagged-1 signal from bone marrow microenvironment is required for endothelial progenitor cell development for neovascularization. *Circulation* 118: 157–165, 2008.
87. Kwon SM, Suzuki T, Kawamoto A, Ii M, Eguchi M, Akimaru H, Wada M, Matsumoto T, Masuda H, Nakagawa Y, Nishimura H, Kawai K, Takaki S, and Asahara T. Pivotal role of Ink adaptor protein in endothelial progenitor cell biology for vascular regeneration. *Circ Res* 104: 969–977, 2009.
88. Lara-Hernandez R, Lozano-Vilardell P, Blanes P, Torreguitart-Mirada N, Galmes A, and Besalduch J. Safety and efficacy of therapeutic angiogenesis as a novel treatment in patients with critical limb ischemia. *Ann Vasc Surg* 24: 287–294, 2010.
89. Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, and Langer R. Endothelial cells derived from human embryonic stem cells. *Proc Natl Acad Sci U S A* 99: 4391–4396, 2002.
90. Li ZQ, Zhang M, Jing YZ, Zhang WW, Liu Y, Cui LJ, Yuan L, Liu XZ, Yu X, and Hu TS. The clinical study of autologous peripheral blood stem cell transplantation by intracoronary infusion in patients with acute myocardial infarction (AMI). *Int J Cardiol* 115: 52–56, 2007.
91. Lin Y, Weisdorf DJ, Solovey A, and Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest* 105: 71–77, 2000.
92. Liu L, Liu H, Jiao J, Bergeron A, Dong JF, and Zhang J. Changes in circulating human endothelial progenitor cells after brain injury. *J Neurotrauma* 24: 936–943, 2007.
93. Llevadot J, Murasawa S, Kureishi Y, Uchida S, Masuda H, Kawamoto A, Walsh K, Isner JM, and Asahara T. HMG-CoA reductase inhibitor mobilizes bone marrow—derived endothelial progenitor cells. *J Clin Invest* 108: 399–405, 2001.
94. Losordo DW and Kishore R. A big promise from the very small identification of circulating embryonic stem-like pluripotent cells in patients with acute myocardial infarction. *J Am Coll Cardiol* 53: 10–12, 2009.
95. Losordo DW, Schatz RA, White CJ, Udelson JE, Veereshwarayya V, Durgin M, Poh KK, Weinstein R, Kearney M, Chaudhry M, Burg A, Eaton L, Heyd L, Thorne T, Shturman L, Hoffmeister P, Story K, Zak V, Dowling D, Traverse JH, Olson RE, Flanagan J, Sodano D, Murayama T, Kawamoto A, Kusano KF, Wollins J, Welt F, Shah P, Soukas P, Asahara T, and Henry TD. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation* 115: 3165–3172, 2007.
96. Lu X, Proctor SJ, and Dickinson AM. The effect of cryopreservation on umbilical cord blood endothelial progenitor cell differentiation. *Cell Transplant* 17: 1423–1428, 2008.
97. Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R, and Rafii S. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med* 7: 1194–1201, 2001.
98. Magen E, Feldman A, Cohen Z, Alon DB, Minz E, Chernyavsky A, Linov L, Mishal J, Schlezinger M, and Sthoeger Z. Circulating endothelial progenitor cells, Th1/Th2/Th17-related cytokines, and endothelial dysfunction in resistant hypertension. *Am J Med Sci* 339: 117–122, 2010.
99. Marsboom G, Pokreisz P, Gheysens O, Vermeersch P, Gillijns H, Pellens M, Liu X, Collen D, and Janssens S. Sustained endothelial progenitor cell dysfunction after chronic hypoxia-induced pulmonary hypertension. *Stem Cells* 26: 1017–1026, 2008.
100. Massa M, Rosti V, Ferrario M, Campanelli R, Ramajoli I, Rosso R, De Ferrari GM, Ferlini M, Goffredo L, Bertoletti A, Klersy C, Pecci A, Moratti R, and Tavazzi L. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood* 105: 199–206, 2005.
101. Masuda H, Kalka C, Takahashi T, Yoshida M, Wada M, Kobori M, Itoh R, Iwaguro H, Eguchi M, Iwami Y, Tanaka R, Nakagawa Y, Sugimoto A, Ninomiya S, Hayashi S, Kato S, and Asahara T. Estrogen-mediated endothelial progenitor cell biology and kinetics for physiological postnatal vasculogenesis. *Circ Res* 101: 598–606, 2007.

102. Matsumoto T, Kawamoto A, Kuroda R, Ishikawa M, Mifune Y, Iwasaki H, Miwa M, Horii M, Hayashi S, Oyamada A, Nishimura H, Murasawa S, Doita M, Kurosaka M, and Asahara T. Therapeutic potential of vasculogenesis and osteogenesis promoted by peripheral blood CD34-positive cells for functional bone healing. *Am J Pathol* 169: 1440–1457, 2006.
103. Mauro E, Rigolin GM, Fraulini C, Sofritti O, Ciccone M, De Angeli C, Castoldi G, and Cuneo A. Mobilization of endothelial progenitor cells in patients with hematological malignancies after treatment with filgrastim and chemotherapy for autologous transplantation. *Eur J Haematol* 78: 374–380, 2007.
104. Mayr FB, Spiel AO, Leitner JM, Firbas C, Sieghart W, and Jilma B. Effects of low dose endotoxemia on endothelial progenitor cells in humans. *Atherosclerosis* 195: e202–e206, 2007.
105. Michaud SE, Dussault S, Haddad P, Groleau J, and Rivard A. Circulating endothelial progenitor cells from healthy smokers exhibit impaired functional activities. *Atherosclerosis* 187: 423–432, 2005.
106. Mifune Y, Matsumoto T, Kawamoto A, Kuroda R, Shoji T, Iwasaki H, Kwon SM, Miwa M, Kurosaka M, and Asahara T. Local delivery of granulocyte colony stimulating factor-mobilized CD34-positive progenitor cells using bioscaffold for modality of unhealing bone fracture. *Stem Cells* 26: 1395–1405, 2008.
107. Miyamoto Y, Suyama T, Yashita T, Akimaru H, and Kurata H. Bone marrow subpopulations contain distinct types of endothelial progenitor cells and angiogenic cytokine-producing cells. *J Mol Cell Cardiol* 43: 627–635, 2007.
108. Murayama T, Tepper OM, Silver M, Ma H, Losordo DW, Isner JM, Asahara T, and Kalka C. Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization *in vivo*. *Exp Hematol* 30: 967–972, 2002.
109. Murohara T, Ikeda H, Duan J, Shintani S, Sasaki K, Eguchi H, Onitsuka I, Matsui K, and Imaizumi T. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. *J Clin Invest* 105: 1527–1536, 2000.
110. Nagaya N, Kangawa K, Kanda M, Uematsu M, Horio T, Fukuyama N, Hino J, Harada-Shiba M, Okumura H, Tabata Y, Mochizuki N, Chiba Y, Nishioka K, Miyatake K, Asahara T, Hara H, and Mori H. Hybrid cell-gene therapy for pulmonary hypertension based on phagocytosing action of endothelial progenitor cells. *Circulation* 108: 889–895, 2003.
111. Nakajima M, Ogawa M, Shimoda Y, Hiraoka S, Iida M, Koseki H, Shirasawa T, and Furukawa K. Presenilin-1 controls the growth and differentiation of endothelial progenitor cells through its beta-catenin-binding region. *Cell Biol Int* 30: 239–243, 2006.
112. Neumuller J, Neumuller-Guber SE, Lipovac M, Mosgoeller W, Vetterlein M, Pavelka M, and Huber J. Immunological and ultrastructural characterization of endothelial cell cultures differentiated from human cord blood derived endothelial progenitor cells. *Histochem Cell Biol* 126: 649–664, 2006.
113. Nguyen VA, Furhapter C, Obexer P, Stossel H, Romani N, and Sepp N. Endothelial cells from cord blood CD133+ CD34+ progenitors share phenotypic, functional and gene expression profile similarities with lymphatics. *J Cell Mol Med* 13: 522–534, 2009.
114. Nonaka-Sarukawa M, Yamamoto K, Aoki H, Nishimura Y, Tomizawa H, Ichida M, Eizawa T, Muroi K, Ikeda U, and Shimada K. Circulating endothelial progenitor cells in congestive heart failure. *Int J Cardiol* 119: 344–348, 2007.
115. Oliveras A, Soler MJ, Martinez-Estrada OM, Vazquez S, Marco-Feliu D, Vila JS, Vilaro S, and Lloveras J. Endothelial progenitor cells are reduced in refractory hypertension. *J Hum Hypertens* 22: 183–190, 2008.
116. Ott I, Keller U, Knoedler M, Gotze KS, Doss K, Fischer P, Urlbauer K, Debus G, von Bubnoff N, Rudelius M, Schomig A, Peschel C, and Oostendorp RA. Endothelial-like cells expanded from CD34+ blood cells improve left ventricular function after experimental myocardial infarction. *FASEB J* 19: 992–994, 2005.
117. Patschan D, Krupincza K, Patschan S, Zhang Z, Hamby C, and Goligorsky MS. Dynamics of mobilization and homing of endothelial progenitor cells after acute renal ischemia: modulation by ischemic preconditioning. *Am J Physiol Renal Physiol* 291: F176–F185, 2006.
118. Paul JD, Powell TM, Thompson M, Benjamin M, Rodrigo M, Carlow A, Annavajjhala V, Shiva S, Dejam A, Gladwin MT, McCoy JP, Zalos G, Press B, Murphy M, Hill JM, Csako G, Waclawiw MA, and Cannon RO 3rd. Endothelial progenitor cell mobilization and increased intravascular nitric oxide in patients undergoing cardiac rehabilitation. *J Cardiopulm Rehabil Prev* 27: 65–73, 2007.
119. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, and Rafii S. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 95: 952–958, 2000.
120. Pirro M, Schillaci G, Menecali C, Bagaglia F, Paltriccia R, Vaudo G, Mannarino MR, and Mannarino E. Reduced number of circulating endothelial progenitors and HOXA9 expression in CD34+ cells of hypertensive patients. *J Hypertens* 25: 2093–2099, 2007.
121. Povsic TJ, Zavodni KL, Kelly FL, Zhu S, Goldschmidt-Clermont PJ, Dong C, and Peterson ED. Circulating progenitor cells can be reliably identified on the basis of aldehyde dehydrogenase activity. *J Am Coll Cardiol* 50: 2243–2248, 2007.
122. Powell TM, Paul JD, Hill JM, Thompson M, Benjamin M, Rodrigo M, McCoy JP, Read EJ, Khuu HM, Leitman SF, Finkel T, and Cannon RO 3rd. Granulocyte colony-stimulating factor mobilizes functional endothelial progenitor cells in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 25: 296–301, 2005.
123. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, and Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. *Br J Haematol* 115: 186–194, 2001.
124. Rehman J, Li J, Orschell CM, and March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 107: 1164–1169, 2003.
125. Rodriguez-Losada N, Garcia-Pinilla JM, Jimenez-Navarro MF, and Gonzalez FJ. Endothelial progenitor cells in cell-based therapy for cardiovascular disease. *Cell Mol Biol (Noisy-le-grand)* 54: 11–23, 2008.
126. Rohde E, Bartmann C, Schallmoser K, Reinisch A, Lanzer G, Linkesch W, Guelly C, and Strunk D. Immune cells mimic the morphology of endothelial progenitor colonies *in vitro*. *Stem Cells* 25: 1746–1752, 2007.

127. Rohde E, Malischnik C, Thaler D, Maierhofer T, Linkesch W, Lanzer G, Guelly C, and Strunk D. Blood monocytes mimic endothelial progenitor cells. *Stem Cells* 24: 357–367, 2006.
128. Rosti V, Massa M, Campanelli R, De Amici M, Piccolo G, and Perfetti V. Vascular endothelial growth factor promoted endothelial progenitor cell mobilization into the peripheral blood of a patient with POEMS syndrome. *Haematologica* 92: 1291–1292, 2007.
129. Sarto P, Balducci E, Balconi G, Fiordaliso F, Merlo L, Tuzzato G, Pappagallo GL, Frigato N, Zanocco A, Forestieri C, Azzarello G, Mazzucco A, Valenti MT, Alborino F, Noventa D, Vinante O, Pascotto P, Sartore S, Dejana E, and Latini R. Effects of exercise training on endothelial progenitor cells in patients with chronic heart failure. *J Card Fail* 13: 701–708, 2007.
130. Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, Abolmaali ND, Vogl TJ, Hofmann WK, Martin H, Dimmeler S, and Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol* 44: 1690–1699, 2004.
131. Schatteman GC, Hanlon HD, Jiao C, Dodds SG, and Christy BA. Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. *J Clin Invest* 106: 571–578, 2000.
132. Schmidt-Lucke C, Rossig L, Fichtlscherer S, Vasa M, Britten M, Kamper U, Dimmeler S, and Zeiher AM. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 111: 2981–2987, 2005.
133. Segal MS, Shah R, Afzal A, Perrault CM, Chang K, Schuler A, Beem E, Shaw LC, Li Calzi S, Harrison JK, Tran-Son-Tay R, and Grant MB. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. *Diabetes* 55: 102–109, 2006.
134. Senegaglia AC, Barboza LA, Dallagiovanna B, Aita CA, Hansen P, Rebelatto CL, Aguiar AM, Miyague NI, Shigunov P, Barchiki F, Correa A, Olandoski M, Krieger MA, and Brofman PR. Are purified or expanded cord blood-derived CD133+ cells better at improving cardiac function? *Exp Biol Med (Maywood)* 235: 119–129, 2010.
135. Senegaglia AC, Brofman PR, Aita CA, Dallagiovanna B, Rebelatto CL, Hansen P, Barchiki F, and Krieger MA. *In vitro* formation of capillary tubules from human umbilical cord blood cells with perspectives for therapeutic application. *Rev Bras Cir Cardiovasc* 23: 467–473, 2008.
136. Sharpe EE 3rd, Teleron AA, Li B, Price J, Sands MS, Alford K, and Young PP. The origin and *in vivo* significance of murine and human culture-expanded endothelial progenitor cells. *Am J Pathol* 168: 1710–1721, 2006.
137. Shepherd RM, Capoccia BJ, Devine SM, Dipersio J, Trinkaus KM, Ingram D, and Link DC. Angiogenic cells can be rapidly mobilized and efficiently harvested from the blood following treatment with AMD3100. *Blood* 108: 3662–3667, 2006.
138. Shimura K, Ashihara E, Shimazaki C, Matsunaga S, Taniguchi K, Uchiyama H, Matsumoto Y, Kimura S, Matsubara H, Taniwaki M, and Maekawa T. Circulating endothelial progenitor cells decreased in patients with sclerodermatous chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 14: 426–437, 2008.
139. Shintani S, Kusano K, Ii M, Iwakura A, Heyd L, Curry C, Wecker A, Gavin M, Ma H, Kearney M, Silver M, Thorne T, Murohara T, and Losordo DW. Synergistic effect of combined intramyocardial CD34+ cells and VEGF2 gene therapy after MI. *Nat Clin Pract Cardiovasc Med* 3 Suppl 1: S123–S128, 2006.
140. Shmilovich H, Deutsch V, Roth A, Miller H, Keren G, and George J. Circulating endothelial progenitor cells in patients with cardiac syndrome X. *Heart* 93: 1071–1076, 2007.
141. Smadja DM, Basire A, Amelot A, Conte A, Bieche I, Le Bonniec BF, Aiach M, and Gaussem P. Thrombin bound to a fibrin clot confers angiogenic and hemostatic properties on endothelial progenitor cells. *J Cell Mol Med* 12: 975–986, 2008.
142. Smadja DM, Bieche I, Helley D, Laurendeau I, Simonin G, Muller L, Aiach M, and Gaussem P. Increased VEGFR2 expression during human late endothelial progenitor cells expansion enhances *in vitro* angiogenesis with up-regulation of integrin alpha(6). *J Cell Mol Med* 11: 1149–1161, 2007.
143. Sozer S, Ishii T, Fiel MI, Wang J, Wang X, Zhang W, Godbold J, Xu M, and Hoffman R. Human CD34+ cells are capable of generating normal and JAK2V617F positive endothelial like cells *in vivo*. *Blood Cells Mol Dis* 43: 304–312, 2009.
144. Stamm C, Kleine HD, Choi YH, Dunkelmann S, Lauffs JA, Lorenzen B, David A, Liebold A, Nienaber C, Zurakowski D, Freund M, and Steinhoff G. Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. *J Thorac Cardiovasc Surg* 133: 717–725, 2007.
145. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber CA, Freund M, and Steinhoff G. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 361: 45–46, 2003.
146. Steiner S, Niessner A, Ziegler S, Richter B, Seidinger D, Pleiner J, Penka M, Wolzt M, Huber K, Wojta J, Minar E, and Kopp CW. Endurance training increases the number of endothelial progenitor cells in patients with cardiovascular risk and coronary artery disease. *Atherosclerosis* 181: 305–310, 2005.
147. Strehlow K, Werner N, Berweiler J, Link A, Dirnagl U, Priller J, Laufs K, Ghaeni L, Milosevic M, Bohm M, and Nickenig G. Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. *Circulation* 107: 3059–3065, 2003.
148. Sturiale A, Coppolino G, Loddo S, Criseo M, Campo S, Crasci E, Bolignano D, Nostro L, Teti D, and Buemi M. Effects of haemodialysis on circulating endothelial progenitor cell count. *Blood Purif* 25: 242–251, 2007.
149. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, and Asahara T. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 5: 434–438, 1999.
150. Tamaki T, Akatsuka A, Ando K, Nakamura Y, Matsuzawa H, Hotta T, Roy RR, and Edgerton VR. Identification of myogenic-endothelial progenitor cells in the interstitial spaces of skeletal muscle. *J Cell Biol* 157: 571–577, 2002.
151. Tanaka R, Wada M, Kwon SM, Masuda H, Carr J, Ito R, Miyasaka M, Warren SM, Asahara T, and Tepper OM. The effects of flap ischemia on normal and diabetic progenitor cell function. *Plast Reconstr Surg* 121: 1929–1942, 2008.
152. Tatsumi T, Ashihara E, Yasui T, Matsunaga S, Kido A, Sasada Y, Nishikawa S, Hadase M, Koide M, Nakamura R, Irie H, Ito K, Matsui A, Matsui H, Katamura M, Kusuoka S, Matoba S, Okayama S, Horii M, Uemura S, Shimazaki C, Tsuji H, Saito Y, and Matsubara H. Intracoronary trans-

plantation of non-expanded peripheral blood-derived mononuclear cells promotes improvement of cardiac function in patients with acute myocardial infarction. *Circ J* 71: 1199–1207, 2007.
153. Thum T, Hoeber S, Froese S, Klink I, Stichtenoth DO, Galuppo P, Jakob M, Tsikas D, Anker SD, Poole-Wilson PA, Borlak J, Ertl G, and Bauersachs J. Age-dependent impairment of endothelial progenitor cells is corrected by growth-hormone-mediated increase of insulin-like growth-factor-1. *Circ Res* 100: 434–443, 2007.
154. Thum T, Tsikas D, Stein S, Schultheiss M, Eigenthaler M, Anker SD, Poole-Wilson PA, Ertl G, and 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* 46: 1693–1701, 2005.
155. Timmermans F, Plum J, Yoder MC, Ingram DA, Vandekerckhove B, and Case J. Endothelial progenitor cells: identity defined? *J Cell Mol Med* 13: 87–102, 2009.
156. Tobler K, Freudenthaler A, Baumgartner-Parzer SM, Wolzt M, Ludvik B, Nansalmaa E, Nowotny PJ, Seidinger D, Steiner S, Luger A, and Artwohl M. Reduction of both number and proliferative activity of human endothelial progenitor cells in obesity. *Int J Obes (Lond)* 34: 687–700, 2010.
157. Urao N, Inomata H, Razvi M, Kim HW, Wary K, McKinney R, Fukai T, and Ushio-Fukai M. Role of nox2-based NADPH oxidase in bone marrow and progenitor cell function involved in neovascularization induced by hindlimb ischemia. *Circ Res* 103: 212–220, 2008.
158. Valgimigli M, Rigolin GM, Fucili A, Porta MD, Soukhomovskaia O, Malagutti P, Bugli AM, Bragotti LZ, Francolini G, Mauro E, Castoldi G, and Ferrari R. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. *Circulation* 110: 1209–1212, 2004.
159. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, and Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 89: E1–E7, 2001.
160. Wang CH, Ting MK, Verma S, Kuo LT, Yang NI, Hsieh IC, Wang SY, Hung A, and Cherng WJ. Pioglitazone increases the numbers and improves the functional capacity of endothelial progenitor cells in patients with diabetes mellitus. *Am Heart J* 152: 1051.e1–1051.e8, 2006.
161. Wang CH, Verma S, Hsieh IC, Chen YJ, Kuo LT, Yang NI, Wang SY, Wu MY, Hsu CM, Cheng CW, and Cherng WJ. Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. *J Mol Cell Cardiol* 41: 34–43, 2006.
162. Werner C, Kamani CH, Gensch C, Bohm M, and Laufs U. The peroxisome proliferator-activated receptor-gamma agonist pioglitazone increases number and function of endothelial progenitor cells in patients with coronary artery disease and normal glucose tolerance. *Diabetes* 56: 2609–2615, 2007.
163. Wu X, Rabkin-Aikawa E, Guleserian KJ, Perry TE, Masuda Y, Sutherland FW, Schoen FJ, Mayer JE Jr., and Bischoff J. Tissue-engineered microvessels on three-dimensional biodegradable scaffolds using human endothelial progenitor cells. *Am J Physiol Heart Circ Physiol* 287: H480–H487, 2004.
164. Xia L, Zhou XP, Zhu JH, Xie XD, Zhang H, Wang XX, Chen JZ, and Jian S. Decrease and dysfunction of endothelial progenitor cells in umbilical cord blood with maternal preeclampsia. *J Obstet Gynaecol Res* 33: 465–474, 2007.
165. Yamada Y and Takakura N. Physiological pathway of differentiation of hematopoietic stem cell population into mural cells. *J Exp Med* 203: 1055–1065, 2006.
166. Yamamoto K, Kondo T, Suzuki S, Izawa H, Kobayashi M, Emi N, Komori K, Naoe T, Takamatsu J, and Murohara T. Molecular evaluation of endothelial progenitor cells in patients with ischemic limbs: therapeutic effect by stem cell transplantation. *Arterioscler Thromb Vasc Biol* 24: e192–e196, 2004.
167. Yip HK, Chang LT, Chang WN, Lu CH, Liou CW, Lan MY, Liu JS, Youssef AA, and Chang HW. Level and value of circulating endothelial progenitor cells in patients after acute ischemic stroke. *Stroke* 39: 69–74, 2008.
168. Yu D, Sun X, Qiu Y, Zhou J, Wu Y, Zhuang L, Chen J, and Ding Y. Identification and clinical significance of mobilized endothelial progenitor cells in tumor vasculogenesis of hepatocellular carcinoma. *Clin Cancer Res* 13: 3814–3824, 2007.

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Abbreviations Used

AMI = acute myocardial infarction
Ang-1 = angiopoietin-1
AP = angina pectoris
BDNF = brain-derived neurotrophic factor
BM = bone marrow
CB = cord blood
CFA = colony forming assay
CLI = chronic limb ischemia
EC = endothelial cell
eNOS = endothelial nitric oxide synthase
EOC = endothelial outgrowth cell
EPC = endothelial progenitor cell
ES = embryonic stem
FACS = fluorescence-activated cell sorter
HSC = hematopoietic stem cell
MACS = magnetically activated cell sorting
MMP = matrix metalloproteinase
MNC = mononuclear cell
NA = not available
NO = nitric oxide
NRT = nonrandomized trial
NUF = nonunion fracture
OMI = old myocardial infarction
PB = peripheral blood
RMI = recent myocardial infarction
RT = randomized trial
T/C = treatment/control
UCB = umbilical cord blood
VEGF = vascular endothelial growth factor

血管老化からみた Stem cell aging

酸化ストレスと血管内皮前駆細胞の老化

Oxidative stress and endothelial progenitor cell senescence

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Key Words:endothelial progenitor cell, senescence, oxidative stress, 血管内皮前駆細胞，老化，酸化ストレス

Abstract

近年，糖尿病，高脂血症，高血圧症，喫煙，肥満等の心血管危険因子を有する患者において末梢血中血管内皮前駆細胞(Endothelial Progenitor Cell=EPC)の数及び機能低下との因果関係が報告され，EPCが血管のホメオスターシス維持機構にも重要な役割を担っており酸化ストレスによるEPC機能低下が血管のホメオスターシス維持の破綻をきたし動脈硬化の進行及び虚血性疾患の発症に至ると考えられる。この意味においてEPCは新たな心血管危険因子におけるバイオマーカーとして診断的有用性が期待される。一方，心血管危険因子がもとで活性酸素種の産生亢進により慢性的酸化ストレスへの暴露が原因でEPCが機能を発揮する上で重要な細胞内シグナル機構の破綻，老化促進シグナルの活性化が解明されつつあり，今後，これらのシグナルを制御するEPC機能改善，老化抑制作用を有する薬剤や因子による治療や新薬の開発が期待される。さらに虚血性疾患患者に対する自己EPC移植療法とそれらの薬剤との併用療法による血管再生療法の開発，有効性向上が期待される。

■はじめに

骨髄由来の血管内皮前駆細胞(Endothelial Progenitor Cell=EPC)は，血管再生能を有する細胞として1997年に発見された。以来，その血管生物学的動態解明の研究が盛んに行われ，虚血性心血管疾患患者を対象として自己EPC移植療法が臨床応用されている。近年，EPCの細胞特性として血管再生能のみならず生体内血管修復能による血管機能のホメオスターシス維持にも関与すると考えられる。このようなEPCの機能は，加齢，糖尿病，高血圧症，高脂質血症，肥満等の慢性的な酸化ストレスにより傷害され，EPCのアポトーシスや老化が促進されることが判明している。EPCの細胞レベルでの老化による機能低下は，成体における血管形成能，再生能の低下のみならず，生理的血管機能の低下を引き起こし動脈硬化が促進され，虚血性心血管疾患の顕在化に至る。本稿では，酸化ストレスによるEPCの老化の機序，現行の自己EPC移植療法の問題点，さらに考案される抗酸化ストレスEPC老化抑制療法につき述べる。

■酸化ストレスとEPC老化

1）EPCの細胞生物学的特性

従来骨髄由来血液幹細胞として認識されていた末梢血CD34陽性細胞がin vitro内皮細胞培養系において，血管網形成能やVEGFに対する増殖能，遊走能を有し，in vivo 虚血動物モデルにおいて本細胞を投与すると局所血管形成部位に集積し，新生血管構築に貢献することから，生体内EPCの存在が明らかとなった[1]。未分化EPCは，VEGF，SDF-1α,

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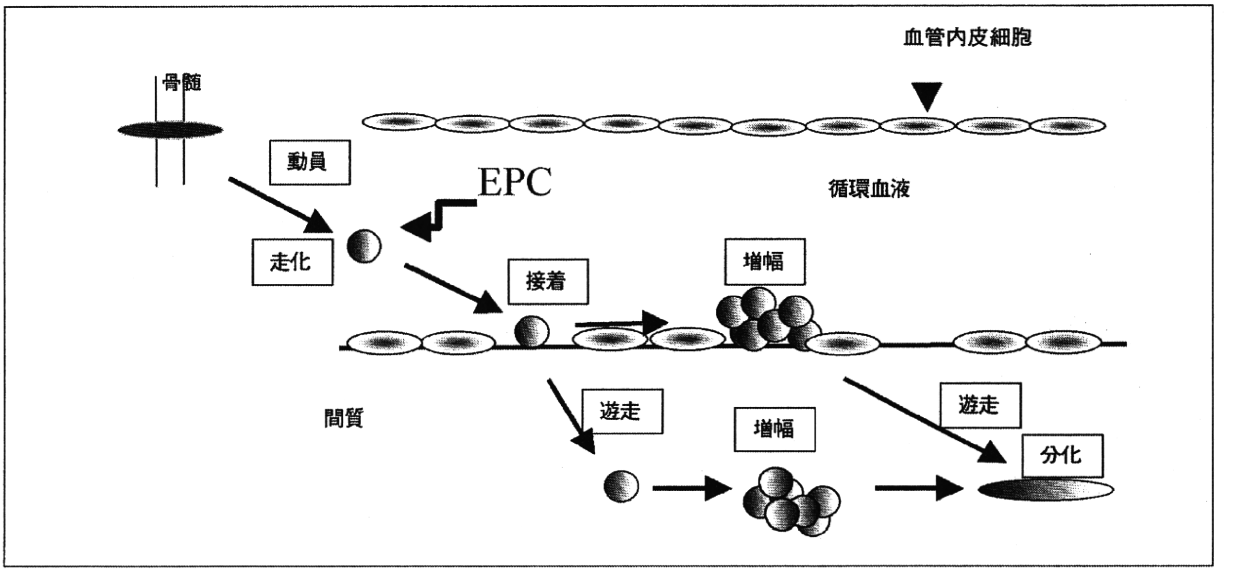

図1　血管内皮前駆細胞の生物活性
骨髄で産生されたEPCは末梢血へ動員され，血管傷害部位や血管再生局所から産生される走化性因子（VEGF, SDF-1等）により血管壁に到達し接着する。その後，遊走，増殖，さらに分化を遂げる。

G-CSF等の血管形成局所から産生されるケモカインや女性ホルモン（エストロゲン）により骨髄から動員され局所に到達し，接着，遊走，さらには増幅，分化を遂げ局所血管内皮細胞と協調して血管形成に貢献する[2]。特に，局所EPCの細胞生物学的特性のなかで増幅能と分化能は新生血管数増加と機能獲得に重要であると考えられる（図1）。

2）酸化ストレスによるEPCの老化機序

EPCの細胞生物活性として動員能，接着能，遊走能，増殖能，分化能が正常に機能することが血管のホメオスターシスや血管再生が円滑かつ効率的に進むためには必須である。しかし，加齢をはじめ，生活習慣病による糖尿病，高血圧症，高脂血症，肥満等のメタボリックシンドロームにおいては，血糖，酸化LDLの他，レムナントリポ蛋白，NADPH酸化酵素等の活性酸素種（ROS）産生促進因子が過剰となり，EPCは蓄積したROSによる酸化ストレスに暴露される[3, 4]。その結果，EPC細胞活性が傷害されることになる。すなわち『EPCの老化』とは，上記の原因によって産生され細胞内に蓄積されたROSによりEPCの細胞活性が正常に発揮されなくなった現象と定義される。その結果，生体内の血管修復能および血管ホメオスターシスが破綻し，動脈硬化，虚血性疾患に至ると考えられる。

ROSは，EPC細胞活性において主役的役割を担うPI-3キナーゼ/Aktシグナル経路の活性化を抑制し，その結果，eNOSの活性化が抑制されることがEPC細胞活性の低下をもたらすと考えられる[5]。また近年，EPCにおいても，このPI-3キナーゼ/Aktシグナル経路は細胞老化抑制のテロメア伸長反応を担うテロメラーゼ活性に重要であることが判明しており[6~8]，酸化ストレスによるこのシグナル活性の低下によりテロメラーゼ活性を低下させ，テロメア短縮，DNA傷害を引き起こす。その結果，細胞分裂が停止しEPCの老化を促進することが考えられる。

一方，PI-3キナーゼ/Aktシグナル経路の持続的活性化はEPCの酸素消費を亢進しROSの細胞内蓄積を促進し，またスーパーオキサイドディスムターゼ，カタラーゼ等のROSスカベンジャー酵素発現の促進性転写因子FOXOの発現を抑制することにより逆に老化を促進する[5]。このようにPI-3キナーゼ/Aktシグナル経路はEPC老化のシグナルにおける『もろ刃の剣』として重要なシグナルとして位置づけられる。また細胞分裂に関与するサイクリン依存性キナーゼ抑制因子である$p16^{INK4a}$の発現及びリン酸化がROSの作用により亢進し，細胞分裂が停止しEPCの老化及びアポトーシスが促進することもその機序として考えられる[9]（図2）。

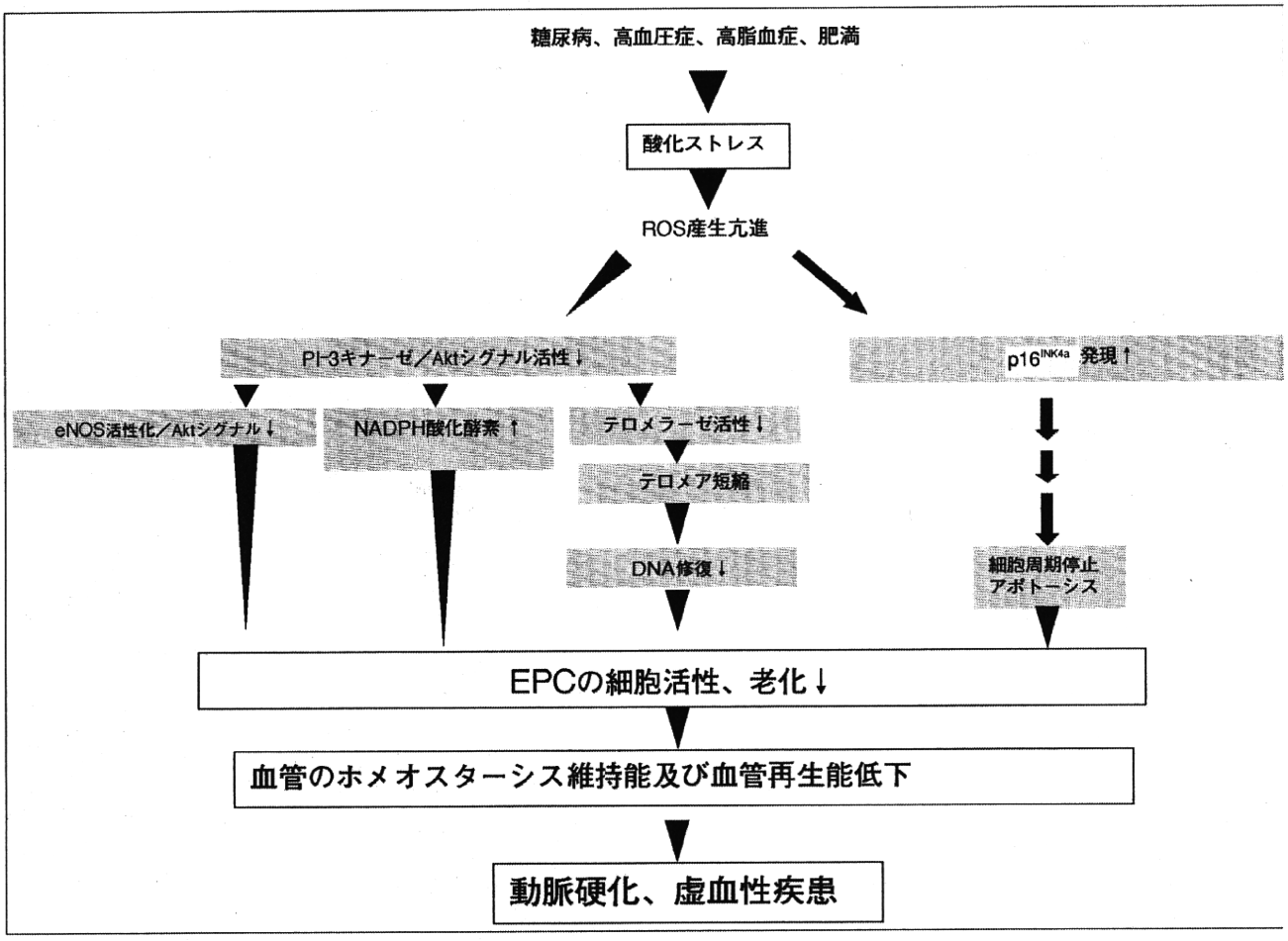

図2　酸化ストレスとEPCの老化機序

図1のEPCの細胞活性が酸化ストレスにより低下しEPCの老化が促進され，PI-3キナーゼ／Aktシグナル活性低下及びサイクリン依存性キナーゼ抑制因子$p16^{INK4a}$の発現亢進により細胞周期が停止しアポトーシスが起こる。これらの細胞内機序によりEPCの細胞活性が低下し老化が惹起される。その結果，血管のホメオスターシス維持能及び血管再生能が障害され，動脈硬化，虚血性疾患に至る。

■EPC移植による血管再生療法の現状及びその問題点

近年，EPCの細胞生物学的特性を基盤に，従来の投薬治療による改善が望めない心血管系虚血性疾患を対象としてEPC移植による血管再生療法が開発された。血管形成に伴う未分化EPCを病変局所へ移植し，EPCの増幅能，分化能を利用し，積極的に機能的血管形成を促進することによる局所血流を改善し組織機能を回復させることが治療概念である。現状では，患者末梢血から採取した未分化EPC（CD34+細胞）を虚血局所に移植する治療が臨床研究として実施され，その安全性は確認され統計学的な有効性は証明されているが[10]，症例ごとの有効性の相違が指摘されている。これは，生活習慣病を基礎疾患に持つ患者において，循環器疾患危険因子である前述の糖尿病[11]，喫煙[12]，高脂質血症[13]，肥満[14]といった酸化ストレスや慢性炎症の環境下では，前述の細胞内老化機序により骨髄からのEPCの動員が減少し，局所におけるEPCの細胞機能が低下すると考えられる。すなわち元々，末梢血単核球中約0.5%以下である希少なEPCがさらに減少し機能低下が認められるので，移植用に採取された自己のEPC数が不十分な場合，あるいは十分量採取できても機能低下により，移植されたEPCによる血管再生治療効果が不十分になると考えられる。

従って今後，EPC移植療法の有効性を確実にするためには，移植EPCが血管形成能を十分に発揮できるようにその細胞機能を改善させる必要がある。

■抗酸化ストレス薬，因子によるEPC機能改善

抗酸化作用薬，因子を用いたROS産生抑制による酸化ストレスの軽減及び除去は，特に循環器領域においてEPCを介する成体内の血管ホメオスターシス維持及び血管修復能の改善や動脈硬化及び心血管虚血性疾患の予防，さらに移植自己EPCそのものの血管再生能の改善に繋がり，EPC移植療法の有効性向上が期待される。抗高脂血症薬のHMG-CoA変換酵素阻害剤（スタチン系薬剤）[15]はPI-3キナーゼ/Aktシグナルを介してeNOSの発現を上昇させEPCの増幅分化を促進させる。また，糖尿病におけるインスリン抵抗性治療薬のペルオキシソーム増殖因子活性化受容体-γ作動薬(PPARγアゴニスト)であるピオグリタゾンは，PI-3キナーゼ/Aktシグナルを介してeNOS発現を上昇させ，NADPH酸化酵素活性を抑制しEPCの増幅分化能を改善させると考えられる[16]。

また抗高血圧薬ではアンギオテンシンII-タイプI受容体拮抗剤はPPARγの発現を上昇させることにより同様の作用を有する[17]。β-ブロッカー や適度な運動[18]はeNOS発現上昇作用，NADPH酸化酵素活性抑制作用によりEPC増幅分化活性を発揮する[18, 19]。アンギオテンシン変換酵素阻害剤は，EPCの動員促進作用が報告されているが[20]，eNOS発現上昇，NADPH酸化酵素活性抑制作用を有することからβ-ブロッカーと同様の作用を有することが示唆される。また，HDLや赤ワインの成分であるポリフェノールのレスベラトロールもPI-3キナーゼ/Aktシグナルを介してeNOSの発現亢進及びテロメラーゼ活性上昇作用によりEPC老化を抑制する[6, 7]。

このような薬剤は，本来の対象疾患への薬効以外に前述の如く生体内酸化ストレスの除去をもたらしEPC機能の改善による抵抗動脈硬化作用，虚血性疾患の発症顕在化予防といった作用が期待される。従って，心血管危険因子のある患者への投薬はこの理由により有用と考えられる。また，EPC移植療法対象患者においても，自己EPC採取前にこれらの薬剤を服薬し自己生体内EPCの機能改善後に，採取したEPCを移植することはEPC移植療法の有効性向上に繋がることが期待される。さらに，テロメラーゼ活性化作用薬，ROSスカベンジャー発現促進薬等も今後，EPC機能改善，老化抑制薬として開発が期待される。

■おわりに

EPCは血管機能のホメオスターシス及び血管修復能に重要な役割を担っている。近年，自然加齢以外に，特に循環器領域において酸化ストレスを惹起する心血管危険因子亢進とEPC機能と数の低下，老化との因果関係が報告されるようになった。今後益々，EPC機能動態は病態を正確に反映するバイオマーカーとして有用になることが期待される。また，さらに有効な抗酸化ストレスによるEPC機能改善療法の開発が期待される。

文　献

1) Asahara T, Masuda H, Takahashi T, *et al.* Circ Res. Aug 6 1999; 85(3): 221-228.
2) Isner JM, Asahara T. J Clin Invest. May 1999;103(9): 1231-1236.
3) Fadini GP, de Kreutzenberg SV, Coracina A, *et al.* Eur Heart J. Sep 2006; 27(18): 2247-2255.
4) Satoh M, Ishikawa Y, Takahashi Y, *et al.* Atherosclerosis. Jun 2008;198(2): 347-353.
5) Nogueira V, Park Y, Chen CC, *et al.* Cancer Cell. Dec 9 2008;14(6): 458-470.
6) Xia L, Wang XX, Hu XS, *et al.* Br J Pharmacol. Oct 2008;155(3): 387-394.
7) Pu DR, Liu L. Med Hypotheses. 2008;70(2): 338-342.
8) Zhu J, Wang X, Shang Y, *et al.* Vascul Pharmacol. Aug-Sep 2008; 49(2-3): 106-110.
9) Yang DG, Liu L, Zheng XY. Ageing Res Rev. Apr 2008;7(2): 137-146.
10) Kawamoto A, Katayama M, Handa N, *et al.* Stem Cells. Nov 2009; 27(11): 2857-2864.
11) Tepper OM, Galiano RD, Capla JM, *et al.* Circulation. Nov 26 2002;106(22): 2781-2786.
12) Michaud SE, Dussault S, Haddad P, *et al.* Atherosclerosis. Aug 2006;187(2): 423-432.
13) Pirro M, Bagaglia F, Paoletti L, *et al.* Ther Adv Cardiovasc Dis. Oct 2008; 2(5): 329-339.
14) Esposito K, Ciotola M, Maiorino MI, *et al.* J Sex Med. Jan 2009; 6(1): 107-114.
15) Dimmeler S, Aicher A, Vasa M, *et al.* J Clin Invest. Aug 2001;108(3): 391-397.
16) Werner C, Kamani CH, Gensch C, *et al.*Diabetes. Oct 2007; 56(10): 2609-2615.
17) Honda A, Matsuura K, Fukushima N, *et al.* Atherosclerosis. Aug 2009; 205(2): 376-384.
18) Yao EH, Fukuda N, Matsumoto T, *et al.* Am J Hypertens. Sep 2008; 21(9): 1062-1068.
19) Jenkins NT, Witkowski S, Spangenburg EE, *et al.* Am J Physiol Heart Circ Physiol. Nov 2009; 297(5): H1798-1805.
20) You D, Cochain C, Loinard C, *et al.* Hypertension. Jun 2008; 51(6): 1537-1544.