Endothelial progenitor cells in age-related vascular remodeling

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Abstract

Accumulating evidence has demonstrated that endothelial progenitor cells (EPCs) could facilitate the reendothelialization of injured arteries by replacing the dysfunctional endothelial cells, thereby suppressing the formation of neointima. Meanwhile, other findings suggest that EPCs may be involved in the pathogenesis of age-related vascular remodeling. This review is presented to summarize the characteristics of EPCs and age-related vascular remodeling. In addition, the role of EPCs in age-related vascular remodeling and possible solutions for improving the therapeutic effects of EPCs in the treatment of age-related diseases are discussed.

Keywords

Endothelial progenitor cell, age, vascular remodeling

Introduction

Aging is characterized by progressive degeneration of tissues and organ systems, aggravation of body functions, and decreasing ability to respond to stress, which increase the risk of age-related diseases¹. Age-related diseases, such as atherosclerosis, hypertension, and type 2 diabetes mellitus, accelerate the process of aging and result in disability and premature death²,³. Among these diseases, atherosclerosis leads to the development of myocardial infarction, sudden cardiac death, ischemic heart disease, and stroke, which are the main causes of morbidity and mortality in the industrialized and some developing countries¹.

Atherosclerosis is considered not only as an age-related disease but also as an age-dependent disease¹. Vascular remodeling, characterized by neointimal hyperplasia, frequently accompanies atherosclerosis⁴. Aggravated vascular remodeling is alleviated by the process of reendothelialization, which occurs by covering the impaired neointimal surface with a functional endothelial monolayer⁵. The endothelial monolayer represents a dynamic structure and functional barrier between the circulating blood and surrounding tissues. It prevents platelet and leukocyte adhesion/aggregation, producing a variety of important vasoregulatory factors such as endothelins and nitric oxide⁶. An imbalance between endothelial cell (EC) damage and repair is the initial step in the development of age-related vascular remodeling⁷. Endothelial repair is accomplished by the migration and proliferation of surrounding mature ECs. However, mature ECs are terminally differentiated with a low proliferative capacity, and their ability to replace the damaged endothelium is altogether limited⁸. Therefore, endothelial repair may need support from other cell types⁸.

Endothelial progenitor cells (EPCs) are currently considered as important contributors to endogenous vascular repair by participating in endothelial regeneration⁹,¹⁰. Studies in animal models and humans have demonstrated that EPCs can facilitate the reendothelialization of injured arteries by replacing dysfunctional ECs, thereby suppressing the formation of neointima¹¹,¹². Meanwhile, other experimental findings have indicated that EPCs may be involved in the pathogenesis of age-related vascular remodeling¹².
This review summarizes the characteristics of EPCs and age-related vascular remodeling. In addition, the role of EPCs in age-related vascular remodeling and possible solutions for improving the therapeutic effects of EPCs in the treatment of age-related diseases are discussed.

Characterization of EPCs

EPCs were initially considered as a group of cells mobilized from the bone marrow that participate in the generation and repair of the vascular endothelium. EPCs have recently been regarded as a heterogeneous population of cells in different stages of maturation, with different origins and several residing sites, such as the spleen, vascular endothelium, and adventitia. EPCs adhere to matrix molecules such as fibronectin, and are positive for both acetylated low-density lipoprotein (acLDL) and Ulex europaeus agglutinin I (UEA-1) lectin. To date, there is no specific marker for identifying EPCs.

Asahara and colleagues reported that circulating CD34+ and fetal liver kinase positive (Flk-1+), also known as vascular endothelial growth factor receptor 2 (VEGFR2) or kinase insert domain receptor (KDR), mononuclear cells (MNCs) may facilitate neo-angiogenesis. These two cell surface markers were the first putative markers proposed for EPC identification. Then, CD34, VEGFR2, and CD133 were used to characterize EPCs, and these biomarkers are the most commonly used surface markers for defining an EPC population.

EPCs consist of two different subpopulations: early-outgrowth and late-outgrowth EPCs. Early-outgrowth EPCs are also termed "circulatory angiogenic cells" (CACs) or "colony forming unit endothelial cells (CFU-EC)", and are adherent spindle-shaped cells that develop after 4–7 days, die after 4 weeks, and have very low proliferative ability. Early-outgrowth EPCs express some surface markers characteristic of progenitor cells, including CD133 and CD34, the endothelial markers CD31 and von Willebrand factor (vWF), the pan-leukocyte marker CD45, and the monocyte marker CD14. Early-outgrowth EPCs lack impressive replicative ability but are prolific producers of several growth factors and cytokines, including VEGF, hepatocyte growth factor (HGF), granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), and interleukin (IL)-8. Early-outgrowth EPCs cannot form a vascular network in vitro, but can adhere to mature ECs, and promote network formation and repair injured ECs through a paracrine mechanism. Late-outgrowth EPCs, also termed "endothelial outgrowth cells" (EOCs) or "endothelial colony forming cells" (ECFCs), display a cobblestone morphology, and start to proliferate and differentiate into mature ECs after 2–3 weeks. These cells express endothelial markers such as KDR, VE-cadherin, and CD146. Late-outgrowth EPCs can improve angiogenesis directly by incorporating into neovessels and further differentiating into mature ECs.

Early- and late-outgrowth EPCs may originate from different angiogenic cell types. Hill et al. developed a colony forming assay based on MNC culture on fibronectin-coated plates, using culture medium that was designed to promote endothelial lineage cell proliferation. These cells (colony forming unit endothelial cells, CFU-ECs, or CFU-Hill) emerged from the cultured non-adherent human peripheral blood MNCs after 48 h of preplating on fibronectin-coated dishes. Hill et al. identified colonies composed of multiple thin flat cells emanating from a central cluster of rounded cells. It was apparent that CFU-ECs contain various blood cells, including hematopoietic progenitor cells, monocytes, and lymphocytes.

Recently, Malinverno et al. identified a subpopulation of vessel-associated ECs with the characteristics of progenitor cells. These PW1-positive cells are highly proliferative and form colonies when cultured at clonal dilution. PW1-positive cells can proliferate to efficiently form new vessels in vivo.

Age-related vascular remodeling

Aging refers to the biological and physiological processes that involve organs, tissues, and cells throughout life, gradually causing a decline of normal functions. Aging is one of the main risk factors for the development of cardiovascular diseases (CVD), which might be due to the structural changes that emerge in the systems and organs, such as complicated alterations in the vasculature, with age.

ECs, located at the interface between blood vessels and tissues, stand poised to respond to the environment and modulate the vascular function to maintain homeostasis and host defenses against microbial invaders and injury. Inappropriate signaling from vascular ECs that leads to endothelial dysfunction induces common diseases characterized by arterial remodeling, notably atherosclerosis.

Recently, the concept of endothelial dysfunction has changed from a pure "damage model" to a more dynamic process, where the effects of endothelial repair are outpaced by local injury. Alteration in the damage/repair balance causes endothelial dysfunction, which is considered the main cause of initiation and development of atherosclerosis. In healthy subjects, a low basal level of endothelial turnover has been unveiled. However, acute injury or chronic immunoinflammatory endothelial dysfunction contributes to the loss of anti-thrombotic function as well as enhanced arrest and transmigration of circulating leukocytes. This pathological vascular remodeling gradually leads to redundant sub-endothelial accumulation of lipids and immune cells, neointimal hyperplasia, excessive proliferation of smooth muscle cells (SMCs), matrix deposition, and foam cell formation. Recently, a growing body of studies have highlighted the involvement of myofibroblasts (MFs) in the neointima induced by vascular injury. MFs are derived from adventitial fibroblasts, the transdifferentiation of SMCs...
residing in the tunica media, and ECs through an endothelial–mesenchymal transition. The major functions of MFs are production and modification of extracellular matrix (ECM), secretion of pro-inflammatory and angiogenic factors, and generation of tensile force. MFs contribute not only to the formation of neointima but also to the thickening of tunica media, adventitial fibrosis, and deposition of the ECM, a process that can lead to late lumen stenosis after vascular injury.

Consequently, occlusive atherosclerotic plaques with lumen stenosis of the arterial wall aggravate and clinically lead to chronic distal tissue ischemia, often complicated by acute myocardial infarction. Beyond acute complications, sufficient endothelial regeneration appears to be crucial for attenuating arterial stenosis secondary to injury (e.g. balloon angioplasty or stent placement). It is also thought to prevent endothelial dysfunction and initiation of corresponding atheromatous plaque growth by replacing the injured ECs.

**EPCs and age-related vascular remodeling**

**EPC status in age-related vascular remodeling**

Previous study has shown that the number of circulating EPCs decreases reversibly with aging, especially in patients with coronary artery disease. For example, Scheubel et al. reported an age-related reduction of circulating EPCs in aged patients undergoing coronary artery bypass grafting. The number of EPC-CFU in culture was found to be inversely correlated with cardiovascular risks in adults. Among patients with low, intermediate, and high numbers of CFU-ECs, those with the highest levels were considered to be the healthiest. Indeed, decreased ability of EPCs to proliferate in vitro and to express the endothelial phenotype was associated with the risk factors for coronary artery disease and endothelial dysfunction.

The circulating number of EPCs can serve as a predicting factor for the patient’s outcome. Dysfunctional EPCs may lead to impaired ability to restore endothelial damage. EPC number was found to be a strong and independent negative predictor of atherosclerotic plaque occurrence in the common carotid artery. Meanwhile, it has also been demonstrated that EPC number is reduced with the presence and progression of preclinical atherosclerosis, and the risk factors contribute to a decrease in aortic and femoral sites, but not in carotid circulation. Peripheral arterial disease was associated with lower cell counts of CD34+ and CD34+/VEGFR2. A decrease of EPCs to below 0.0038% of total circulating peripheral blood MNCs represents a six-fold higher risk for the development of CVDs. Coronary artery disease patients with the highest EPC number have the highest likelihood of remaining event-free.

Therefore, EPCs serve as promising biomarkers of cardiovascular health. However, some investigators reported no correlation between EPC subsets and vascular remodeling, with no direct association of EPC number change with CVD progression. Differences in methods used for identifying EPCs may lead to such disparity.

**Involvement of EPCs in age-related vascular remodeling**

Endothelial damage is an important early step in the initiation and development of atherosclerosis, a hallmark of aging. Structural and functional endothelial damage contributing to atherosclerosis is a common event, and endothelial regeneration is critical for maintaining endothelial homeostasis. In the context of regeneration, animal studies have shown that EPCs efficiently contribute to restoring endothelial function and decrease neointimal formation after arterial injury. An adequate homing of EPCs plays a central role in this regenerative arterial remodeling. The process of EPC homing, including mobilization, recruitment, and adhesion, is regulated by key angiogenic chemokines (CXCL1, CXCL7, CXCL12, CCL2) and their respective receptors (CXCR2, CXCR4, CCR2). Hristov et al. showed that CXCR2 is crucial for the homing of circulating EPCs to sites of arterial injury and for endothelial repair. It was also found that rat bone marrow-derived EPC functional activity could be ameliorated by decreasing cellular senescence via AKT/endothelial nitric oxide synthase (eNOS) pathways and improving homing capacity via increasing CXCR4 expression levels. Walter et al. reported that the use of statins increased circulating rat EPCs and promoted adhesion of cultured human EPCs by augmentation of integrin subunits α5, αv, β1, and β3 of human EPCs. Augmentation of integrin receptor expression may thus promote adhesion and enhance homing of EPCs to foci of ischemia or vascular injury. Meanwhile, EPCs play an important role in the neovascularization of ischemic tissue by promoting the formation of new vessels and releasing angiogenic growth factors. Currently, the common clinical concept claims a protective role for EPCs even during the initiation and development of atherosclerosis, further suggesting that EPCs may reflect the endogenous vascular repair ability. Intramyocardial injection by synergistic local co-administration of angiogenic compounds may help to further promote the homing of EPCs and neovascularization after myocardial infarction. The therapeutic effects are exerted even in the chronic stage, when acute inflammation and oxidative stress are attenuated. Kaushal et al. coated vascular grafts with endogenous EPCs and found that EPCs can exert functions similar to arterial ECs, thereby conferring longer vascular-graft survival. Thus, the coating of stents with EPC-attracting peptides or antibodies to capture EPCs in terms of promoting endothelialization and diminishing in-stent stenosis remains an exciting alternative for clinical application.
pathogenesis of atherosclerosis has newly emerged. Interestingly, transplanted EPCs increase the lipid content and decrease collagen amounts in atherosclerotic plaques of Apoe<sup>−/−</sup> mice<sup>68</sup>. Furthermore, higher serum concentrations of IL-6 and monocyte chemoattractant protein-1, and lower serum concentration of IL-10, were found in mice transfused with EPCs<sup>68</sup>. Increased plasma CXCR2 receptor ligands such as CXCL1 and CXCL7 were clinically related to plaque destabilization, while blocking of CXCR2 was associated with a more stable plaque phenotype in experimental models<sup>69–71</sup>. The influx of CXCR2<sup>+</sup> monocyte subsets containing putative endothelial precursors with inflammatory, proteolytic, and angiogenic properties may partly contribute to these findings<sup>55,71</sup>. Vega et al. found that the atherosclerotic plaque secretome promotes EPC proliferation, mobilization, permeability, contraction, and adhesion<sup>72</sup>. Furthermore, the up-regulated expression of proteins that are mostly involved in cell proliferation, migration, and vascular remodeling was observed in the atherosclerotic plaque secretome treated cells<sup>72</sup>. It was also found that increased circulating CD34<sup>+</sup> cells after coronary stenting may serve as an independent risk factor for predicting in-stent restenosis and indicate the involvement of CD34<sup>+</sup> subpopulations in neointimal hyperplasia<sup>73</sup>. Thus, the dual contribution of EPC subpopulations to vascular remodeling in atherosclerosis needs a critical reevaluation<sup>76</sup>.

In the early stage of primary and secondary atherosclerosis after injury, which is characterized by endothelial dysfunction, EPCs (mainly as late-outgrowth EPCs) mobilize to the injured area, penetrate the site of vessel injury, and differentiate into mature ECs. This in turn replaces the dysfunctional endothelium, further avoiding the development of atherosclerosis (Figure 1(a)). Hence, EPCs may provide a circulating pool of cells that could generate a cellular patch at the site of denuding injury or serve as a cellular reservoir to substitute the injured endothelium<sup>28</sup>. In recent years, accumulating evidence has indicated that activation of tissue-resident ECs through paracrine mechanisms may become more crucial for EPC-based neovascularization than direct differentiation and incorporation into the vasculature<sup>74,75</sup>. Early-outgrowth EPCs secrete several cytokines, such as VEGF, HGF, G-CSF, and GM-CSF<sup>20,21</sup>. Therefore, EPCs (mainly as early-outgrowth EPCs) could also repair the injured ECs by secreting growth factors (Figure 1(a)). Advanced atherosclerosis is characterized by redundant sub-endothelial accumulation of lipids and immune cells, neointimal hyperplasia, excessive proliferation of SMCs, matrix deposition, and foam cell formation<sup>18</sup>. It involves widespread mobilization of EPCs associated with that of monocytes in response to inflammatory factors, such as monocyte chemoattractant protein 1, and may promote plaque instability/vascularization<sup>12</sup> (Figure 1(b)). Again, the contribution of EPCs during vascular remodeling in the early and advanced disease stages, as well as primary and secondary atherosclerosis, requires a more careful and critical reevaluation<sup>76</sup>.

In addition, it should be mentioned that aging may also affect the pathways involved in the contribution of EPCs to vascular remodeling. A significant reduction in the expression of CXCR4 was found in the CD34<sup>+</sup> cell population with aging<sup>77</sup>. It was also found that the surface CXCR4 expression on bone marrow-derived cells was significantly reduced in aged mice compared with young mice<sup>78,79</sup>. Xia et al. showed no difference in the surface expression of CXCR4 receptor in EPCs between older and younger men<sup>80</sup>. However, phosphorylation of JAK-2, a downstream signaling of CXCR4, is markedly decreased in EPCs derived from elderly men<sup>80</sup>. It was suggested that bone marrow-derived EPC functional activity could be ameliorated by decreasing cellular senescence and improving homing capacity through increasing CXCR4 expression levels<sup>81</sup>. So, aging may impair the protective effects of EPCs in vascular remodeling via affecting CXCR4-JAK-2 pathways.

**Obstacles and possible solutions**

There is no specific marker for EPCs, and many studies assessing EPCs have included limited analyses regarding the cell phenotypes. The use of an often poorly defined label “progenitor cells” for heterogeneous therapeutic cell subtypes complicates study comparisons, making it quite challenging to reach definitive conclusions concerning their efficacy<sup>81</sup>. Defining and standardizing EPC surface markers are extremely important for comparing more EPC studies<sup>82</sup>. In addition, the methods for culturing EPCs should be more standardized and, if possible, this should be done in a uniform manner.

Convincing evidence has emerged that EPCs from the elderly are impaired in terms of number, function, and survival<sup>83–87</sup>. Clinical application of cardiovascular cell repair therapy showed some limitations in older patients<sup>88,89</sup>. The major obstacles include degradation of functionality of autologous stem or progenitor cells in older individuals, and difficulties in engraftment and survival of transplanted cells in the hostile host microenvironment<sup>7</sup>. EPC-based therapy showed that an increase in the number or function of circulating EPCs may be effective in the treatment of atherosclerotic diseases<sup>80</sup>. However, large-scale use of cell-based therapy was limited due to the poor viability of EPCs after transplantation<sup>90</sup>. Therefore, enhancement strategies to reactivate the proliferation and function of EPCs in aged patients need to be explored urgently<sup>91</sup>.

EPC function enhancement was observed after administration of growth factors such as HGF and insulin-like growth factor (IGF)-1<sup>92,93</sup>. Recombinant bone morphogenetic protein 4 also markedly improved the migration and adhesion capacity of human EPCs<sup>94</sup>. In clinical application, the most feasible method to improve EPC number and function is drug treatment. To date, the most practicable strategies applied in the clinic are pharmacological treatments with anti-hypertensive and anti-hyperglycemic effects<sup>91</sup>. 
Previous studies reported that beta blockers, calcium channel blockers, and angiotensin II receptor antagonists significantly increased EPC counts. In diabetic and non-diabetic patients, pharmacological products, such as anti-diabetic peroxisome proliferator-activated receptor gamma (PPARG) agonists, have been shown to enhance EPC number and function. Other drugs, for example rosuvastatin and cilostazol, also exerted positive effects on circulating EPC levels. As well as Western medicine, traditional Chinese drugs have recently shown beneficial effects on EPC function. Tanshinone IIA may have the potential to protect EPCs against damage induced by tumor necrosis factor-α. Danhong injection, extracted from Radix Salvia miltiorrhiza and Flos Carthamus tinctorius L, is effective in repairing endothelial lesions by mobilizing EPCs.

Although the drug treatment mentioned earlier could increase the number and function of EPCs, adverse effects of the drugs, such as headache, nausea, and asthenia, may also occur, especially in the elderly population. Non-drug therapies may be good choices to enhance EPC function and avoid adverse effects. Interestingly, exercise has direct beneficial effects on EPC number and function in the aged population. Mediterranean diets and black tea exert protective effects on EPC level and function. As concomitant strategies to enhance EPC number and function, lifestyle...
and diet modifications should be strongly encouraged in aged patients.

Recent attempts to improve the number and function of transplanted EPCs with gene modification may facilitate repair of the injured endothelium and accelerate reendothelialization. Transplantation of genetically modified EPCs that overexpress PDGFR-β, β2AR, and CXCRT7, or with reduced Lnk levels, significantly enhanced the vascular repair ability of EPCs, improving the inhibition of adverse remodeling after vascular injury. EPC transplantation combined with gene transfer may be a promising EPC therapeutic strategy in the future for age-related vascular remodeling.

Conclusions

Endothelial damage is a critical early step in the initiation and development of atherosclerosis. EPCs may repair and replace the injured ECs, and avoid initiation and development of atherosclerosis, through differentiating into mature ECs and the release of protective paracrine factors. However, widespread EPC mobilization may, rather, cause plaque instability in advanced atherosclerosis. Selectively controlling the mobilization and homing of EPCs helps to increase their therapeutic potential and avoid promoting the development of atherosclerotic diseases. EPCs from the elderly are impaired in terms of number, function, and survival. Thus, improving the effectiveness of EPC treatments to delay the progression of age-related vascular remodeling and diseases remains an urgent necessity. Drug regimens, gene transfer, lifestyle, and diet modifications are effective approaches, and may constitute promising therapeutic strategies for the treatment of age-related vascular remodeling.

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References

1. Altabas V, Altabas K, Kirigin L. Endothelial progenitor cells (EPCs) in ageing and age-related diseases: how currently available treatment modalities affect EPC biology, atherosclerosis, and cardiovascular outcomes. Mech Ageing Dev. 2016;159:49–62.

2. Tian XL, Li Y. Endothelial cell senescence and age-related vascular diseases. J Genet Genomics. 2014;41(9):485–95.

3. Bao Q, Pan J, Qi H, Wang L, Qian H, Jiang F, Shao Z, Xu F, Tao Z, Ma Q, Nelson P, Hu X. Aging and age-related diseases—from endocrine therapy to target therapy. Mol Cell Endocrinol. 2014;394(1–2):115–18.

4. Libby P. Inflammation in atherosclerosis. Nature. 2002;420(6917):686–74.

5. Carmeliet P, Moons L, Stassen JM, De Mol M, Bouche A, van den Oord JJ, Kockx M, Collen D. Vascular wound healing and neointima formation induced by perivascular electric injury in mice. Am J Pathol. 1997;150(2):761–76.

6. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwartz BS, Barnathan ES, McCrae KR, Hug BA, Schmidt AM, Stern DM. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood. 1998;91(10):3527–61.

7. Madonna R, Novo G, Balistrieri CR. Cellular and molecular basis of the imbalance between vascular damage and repair in ageing and age-related diseases: as biomarkers and targets for new treatments. Mech Ageing Dev. 2016;159:22–30.

8. Hristov M, Weber C. Endothelial progenitor cells in vascular repair and remodeling. Pharmacol Res. 2008;58(2):148–51.

9. Balistrieri CR, Buffa S, Pisano C, Lio D, Ruvolo G, Mazzesi G. Are endothelial progenitor cells the real solution for cardiovascular diseases? Focus on controversies and perspectives. Biomed Res Int. 2015;2015:835934.

10. Kim SW, Kim H, Cho HJ, Lee JU, Levit R, Yoon YS. Human peripheral blood-derived CD31+ cells have robust angiogenic and vasculogenic properties and are effective for treating ischemic vascular disease. J Am Coll Cardiol. 2010;56(7):593–607.

11. Rabelink TJ, de Boer HC, de Koning EJ, van Zonneveld AJ. Endothelial progenitor cells: more than an inflammatory response? Arterioscler Thromb Vasc Biol. 2004;24(5):834–8.

12. Hristov M, Weber C. Ambivalence of progenitor cells in vascular repair and plaque stability. Curr Opin Lipidol. 2008;19(5):491–7.

13. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275(5302):964–7.

14. Yoder MC. Is endothelium the origin of endothelial progenitor cells? Arterioscler Thromb Vasc Biol. 2010;30(6):1094–103.

15. Yang JX, Pan YY, Ge JH, Chen B, Mao W, Qiu YG, Wang XX. Tanshinone II A attenuates TNF-alpha-induced expression of VCAM-1 and ICAM-1 in endothelial progenitor cells by blocking activation of NF-kappaB. Cell Physiol Biochem. 2016;40(1–2):195–206.

16. Yang JX, Chen B, Pan YY, Han J, Chen F, Hu SJ. Zoledronate attenuates angiogenic effects of angiotensin II-stimulated endothelial progenitor cells via RhoA and MAPK signaling. PLoS One. 2012;7(10):e46511.

17. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood. 2000;95(3):952–8.
18. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. Circ Res. 2004;95(4):343–53.

19. Prater DN, Case J, Ingram DA, Yoder MC. Working hypothesis to redefine endothelial progenitor cells. Leukemia. 2007; 21(6):1141–9.

20. Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, Hwang KK, Oh BH, Lee MM, Park YB. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler Thromb Vasc Biol. 2004; 24(2):288–93.

21. Rehman J, Li J, Orschell CM, March KL. Peripheral blood “endothelial progenitor cells” are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation. 2003;107(8):1164–9.

22. Sieweung DP, Buckle A, Celemajer DS, Ng MK. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. J Am Coll Cardiol. 2008;51(6):660–8.

23. Recchioni R, Marcheselli F, Antonicelli R, Lazzarini R, Mensa E, Testa R, Procopio AD. Olivieri F. Physical activity and progenitor cell-mediated endothelial repair in chronic heart failure: is there a role for epigenetics? Mech Ageing Dev. 2016;159:71–80.

24. Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. Arterioscler Thromb Vasc Biol. 2008;28(9):1584–95.

25. Avogaro A, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes: the role of reparatory mechanisms. Diabetes Care. 2011;34(Suppl 2):S285–S290.

26. Lee PS, Poh KK. Endothelial progenitor cells in cardiovascular diseases. World J Stem Cells. 2014;6(3):355–66.

27. Madonna R, De Caterina R. Circulating endothelial progenitor cells: do they live up to their name? Vascul Pharmacol. 2015; 76:67–92.

28. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med. 2003;348(7):593–600.

29. Richardson MR, Yoder MC. Endothelial progenitor cells: quo vadis? J Mol Cell Cardiol. 2011;50(2):266–72.

30. Malinverno M, Corada M, Ferrari R, Formicola L, Marazzi G, Sassoon D, Dejana E. Peg3/PW1 is a marker of a subset of vessel associated endothelial progenitors. Stem Cells. 2017; 35(5):1328–40.

31. Abdelmagid SM, Barbe MF, Safadi FF. Role of inflammation in the aging bones. Life Sci. 2015;123:25–34.

32. Yıldız O. Vascular smooth muscle and endothelial functions in aging. Ann N Y Acad Sci. 2007;1100:353–60.

33. Ferrari AU, Radaelli A, Centola M. Invited review: Aging and the cardiovascular system. J Appl Physiol (1985). 2003;95(6): 2591–7.

34. Gimbrone MA Jr., Garcia-Cardena G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. Cardiovasc Pathol. 2013;22(1):9–15.

35. Heuschen G, Libby P, Gersh B, Yellon D, Bohm M, Lopaschuk G, Opie L. Cardiovascular remodelling in coronary artery disease and heart failure. Lancet. 2014;383(9932):1933–43.

36. Van Craenenbroeck EM, Conraads VM. Mending injured endothelium in chronic heart failure: a new target for exercise training. Int J Cardiol. 2013;166(2):310–4.

37. Dignat-George F, Sampol J. Circulating endothelial cells in vascular disorders: new insights into an old concept. Eur J Haematol. 2000;65(4):215–20.

38. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685–95.

39. Forte A, Della Corte A, De Feo M, Cerasuolo F, Cipollaro M. Role of myofibroblasts in vascular remodelling: focus on restenosis and aneurysm. Cardiovasc Res. 2010;88(3):395–405.

40. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest. 2003;112(12):1776–84.

41. Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med. 2002;8(11):1257–62.

42. Werner N, Wassmann S, Ahlers P, Schiegel T, Kosiol S, Link A, Walenta K, Nickenig G. Endothelial progenitor cells correlate with endothelial function in patients with coronary artery disease. Basic Res Cardiol. 2007;102(6):565–71.

43. Scheubel RJ, Zorn H, Silber RE, Kuss O, Morawietz H, Holtz J, Simm A. Age-dependent depression in circulating endothelial progenitor cells in patients undergoing coronary artery bypass grafting. J Am Coll Cardiol. 2003;42(12):2073–80.

44. Werner N, Kosiol S, Schiegel T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med. 2005;353(10):999–1007.

45. Kiewisz J, Kaczmarek MM, Pawlowska A, Kmiec Z, Stompor T. Endothelial progenitor cells participation in cardiovascular and kidney diseases: a systematic review. Acta Biochim Pol. 2016;63(3):475–82.

46. Schmidt-Lucke C, Rossig L, Fichtlscherer S, Vasa M, Britten J, Zemel B. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. Circulation. 2005;111(22):2981–7.

47. Lau KK, Chan YH, Kiu KH, Li SW, Tam S, Lau CP, Kwong YL, Tse HF. Burden of carotid atherosclerosis in patients with stroke: relationships with circulating endothelial progenitor cells and hypertension. J Hum Hypertens. 2007;21(6):445–51.

48. Fadini GP, de Kreutzenberg S, Albiero M, Corazza C, Pagnin E, Baesso I, Cignarella A, Bolego C, Plebani M, Nardelli GB, Sartore S, Agostini C, Avogaro A. Gender differences in endothelial progenitor cells and cardiovascular risk profile: the role of female estrogens. Arterioscler Thromb Vasc Biol. 2004;24(2):288–93.
50. Hayek SS, MacNamara J, Tahhan AS, Awad M, Yadalam A, Ko YA, Healy S, Hesareiouch I, Ahmed H, Gray B, Sher SS, Ghasemzadeh N, Patel R, Kim J, Waller EK, Quyyumi AA. Circulating progenitor cells identify peripheral arterial disease in patients with coronary artery disease. Circ Res. 2016;119(4):564–71.

51. Sibal L, Aldibbiat A, Agarwal SC, Mitchell G, Oates C, Razvi S, Weaver JU, Shaw JA, Home PD. Circulating endothelial progenitor cells, endothelial function, carotid intima-media thickness and circulating markers of endothelial dysfunction in people with type 1 diabetes without macrovascular disease or microalbuminuria. Diabetologia. 2009;52(8):1464–73.

52. Xiao Q, Kiechl S, Patel S, Oberhollenzer F, Weger S, Mayr A, Metzler B, Reindl M, Hu Y, Willeit J, Xu Q. Endothelial progenitor cells, cardiovascular risk factors, cytokine levels and atherosclerosis—results from a large population-based study. PLoS One. 2007;2(10):e975.

53. Mannarino E, Pirro M. Endothelial injury and repair: a novel theory for atherosclerosis. Angiology 2008;59(Suppl 2):69S–72S.

54. Zhang L, Xu Q. Stem/Progenitor cells in vascular regeneration. Arterioscler Thromb Vasc Biol. 2014;34(6):1114–9.

55. Hristov M, Zernecke A, Bidzhekov K, Liehn EA, Shagdarsuren M, Kaushal S, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW, Rabkin E, Moran AM, Schoen FJ, Atala A, Soker S, Bischoff J, Mayer JE Jr. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. Nat Med. 2001;7(9):1035–40.

56. Aoki J, Serruys PW, van Beuschem 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(10):1574–9.

57. Blindt R, Vogt F, Astafeiva I, Fuch C, Hristov M, Krott N, Seitz B, Kapurniotu A, Kwok C, Dewor M, Bosserhoff AK, Bernhagen J, Hanrath P, Hoffmann R, Weber C. A novel drug-eluting stent coated with an integrin-binding cyclic Arg-Gly-Asp peptide inhibits neointimal hyperplasia by recruiting endothelial progenitor cells. J Am Coll Cardiol. 2006;47(9):1786–95.

58. George J, Afek A, Abashidze A, Shmilovich H, Deutsch V, Kopolovich J, Miller H, Keren G. Transfer of endothelial progenitor and bone marrow cells influences atherosclerotic plaque size and composition in apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol. 2005;25(12):2636–41.

59. Smith C, Damas JK, Otterdal K, Oie E, Sandberg WJ, Yndestad A, Waecher T, Scholz H, Endresen K, Olofsson PS, Halvorsen B, Gullestad L, Frondsson SS, Hansson GK, Aukrust P. Increased levels of neutrophil-activating peptide-2 in acute coronary syndromes: possible role of platelet-mediated vascular inflammation. J Am Coll Cardiol. 2006;48(8):1591–9.

60. Brelad UM, Halvorsen B, Hol J, Oie E, Paulsson-Berne G, Yndestad A, Smith C, Otterdal K, Hedin U, Waecher T, Sandberg WJ, Frondsson SS, Haraldsen G, Gullestad L, Damas JK, Hansson GK, Aukrust P. A potential role of the CXC chemokine GROalpha in atherosclerosis and plaque destabilization: downregulatory effects of statins. Arterioscler Thromb Vasc Biol. 2008;28(5):1005–11.

61. Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA. A leukocyte homologue of the IL-8 receptor CXCR2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. J Clin Invest. 1998;101(2):353–63.

62. Leor J, Marber M. Endothelial progenitors: a new Tower of Babel? J Am Coll Cardiol. 2006;48(8):1588–90.

63. Schuh A, Liehn EA, Sasse A, Hristov M, Sobota R, Kelm M, Merx MW, Weber C. Transplantation of endothelial progenitor cells improves neovascularization and left ventricular function after myocardial infarction in a rat model. Basic Res Cardiol. 2008;103(1):69–77.

64. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med. 2006;355(12):1210–21.

65. Kaushal S, Amiel GE, Gulserian KJ, Shapira OM, Perry T, Sutherland FW, Rabkin E, Moran AM, Schoen FJ, Atala A, Soker S, Bischoff J, Mayer JE Jr. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. Nat Med. 2001;7(9):1035–40.
Bone marrow rejuvenation accelerates re-endothelialization and attenuates intimal hyperplasia after vascular injury in aging mice. Circ J. 2013;77(12):3045–53.

Rehman J. Feeling the elephant of cardiovascular cell therapy. Cell Transplant. 2010;19(12):1635–44.

Zhang M, Malik AB, Rehman J. Endothelial progenitor cells and vascular repair. Curr Opin Hematol. 2014;21(3):224–8.

Hristov M, Zernecke A, Schober A, Weber C. Adult progenitor cells in vascular remodeling during atherosclerosis. Biol Chem. 2008;389(7):837–44.

Hernandez-Lopez C, Varas A, Sacedon R, Martinez VG, Hidalgo L, Valencia J, Zapata AG, Vicente A. The CXCL12/CXCR4 pair in aged human thymus. Neuroimmunomodulation. 2010;17(3):217–20.

Shao H, Xu Q, Wu Q, Ma Q, Salgueiro L, Wang J, Eton D, Webster KA, Yu H. Defective CXCR4 expression in aged bone marrow cells impairs vascular regeneration. J Cell Mol Med. 2011;15(10):2046–56.

Xu Q, Wang J, He J, Zhou M, Adi J, Webster KA, Yu H. Impaired CXCR4 expression and cell engraftment of bone marrow-derived cells from aged atherogenic mice. Atherosclerosis 2011;219(1):92–9.

Xia WH, Li J, Su C, Yang Z, Chen L, Wu F, Zhang YY, Yu BB, Qiu YX, Wang SM, Tao J. Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men. Aging Cell. 2012;11(1):111–9.

Rehman J. Feeling the elephant of cardiovascular cell therapy. Circulation. 2010;121(2):197–9.

Yang J, Yu J, Li D, Yu S, Ke J, Wang L, Wang Y, Qiu Y, Gao X, Zhang J, Huang L. Store-operated calcium entry-activated autophagy protects EPC proliferation via the CAMKK2-MTOR pathway in ox-LDL exposure. Autophagy. 2017;13(1):82–98.

Rurali E, Bassetti B, Perrucci GL, Zanobini M, Malafronte C, Achilli F, Gambini E. BM ageing: implication for cell therapy with EPCs. Mech Ageing Dev. 2016;159:4–13.

Sanada F, Taniyama Y, Azuma I, Iekushi K, Dosaka N, Yokoi T, Koibuchi N, Kusunoki H, Aizawa Y, Morishita R. Hepatocyte growth factor, but not vascular endothelial growth factor, attenuates angiotensin II-induced endothelial progenitor cell senescence. Hypertension. 2009;53(1):77–82.

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, Bauersachs J. Age-dependent impairment of endothelial progenitor cells is corrected by growth-hormone-mediated increase of insulin-like growth-factor-1. Circ Res. 2007;100(3):434–43.

Xia WH, Chen L, Liang JW, Zhang XY, Su C, Tong X, He J, Li Y, Cao Z, Lin XF, Tao J. BMP4/Id2 signaling pathway is a novel therapeutic target for late outgrowth endothelial progenitor cell-mediated endothelial injury repair. Int J Cardiol. 2017;228:796–804.

Pelliccia F, Pasceri V, Cianfrocca C, Vitale C, Speciale G, Gaudio C, Rosano GM, Mercuro G. Angiotensin II receptor antagonism with telmisartan increases number of endothelial progenitor cells in normotensive patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. Atherosclerosis. 2010;210(2):510–5.
97. De Ciuceis C, Rossini C, Tincani A, Airo P, Scarsi M, Agabiti-Rosei C, Ruggeri G, Caimi L, Ricotta D, Agabiti-Rosei E, Rizzoni D. Effect of antihypertensive treatment with lercanidipine on endothelial progenitor cells and inflammation in patients with mild to moderate essential hypertension. Blood Press. 2016;25(6):337–43.

98. Peixiao S, Ningyuan F, Haiya W. Lercanidipine effect on circulating CD34+ progenitor cells in elderly patients: a randomized study. Curr Med Res Opin. 2016;32(suppl 2):9–12.

99. Sun J, Xie J, Kang L, Ferro A, Dong L, Xu B. Amlodipine effect on endothelial progenitor cells attenuates age-related decline in arterial elasticity in healthy men. Int J Cardiol. 2013;165(2):247–54.

100. Guo Y, Peng R, Liu Q, Xu D. Exercise training-induced different improvement profile of endothelial progenitor cells function in mice with or without myocardial infarction. Int J Cardiol. 2016;221:335–41.

101. Marin C, Yubero-Serrano EM, Lopez-Miranda J, Perez-Jimenez F. Endothelial aging associated with oxidative stress can be modulated by a healthy Mediterranean diet. Int J Mol Sci. 2013;14(5):8869–89.

102. Fernandez JM, Rosado-Alvarez D, Da Silva Grigoletto ME, Rangel-Zuniga OA, Landaua-Diaz LL, Caballero-Villarraso J, Lopez-Miranda J, Perez-Jimenez F, Fuentes-Jimenez F. Moderate-to-high-intensity training and a hypocaloric Mediterranean diet enhance endothelial progenitor cells and fitness in subjects with the metabolic syndrome. Clin Sci (Lond). 2012;123(6):361–73.

103. Maiorino MI, Bellastella G, Petrizzo M, Giocchino M, Caputo M, Giugliano D, Esposito K. Effect of a Mediterranean diet on endothelial progenitor cells and carotid intima-media thickness in type 2 diabetes: follow-up of a randomized trial. Eur J Prev Cardiol. 2017;24(4):399–408.

104. Grassi D, Draijer R, Schalkwijk C, Desideri G, D’Angeli A, Francavilla S, Mulder T, Ferri C. Black tea increases circulating endothelial progenitor cells and improves flow mediated dilatation counteracting deleterious effects from a fat load in hypertensive patients: a randomized controlled study. Nutrients. 2016;8(11):727.

105. Ke X, Shu XR, Wu F, Hu QS, Deng BQ, Wang JF, Nie RQ. Overexpression of the beta2AR gene improves function and re-endothelialization capacity of EPCs after arterial injury in nude mice. Stem Cell Res Ther. 2016;7(1):73.

106. Wang H, Yin Y, Li W, Zhao X, Yu Y, Zhu J, Qin Z, Wang Q, Wang K, Lu W, Liu J, Huang L. Over-expression of PDGFR-beta promotes PDGF-induced proliferation, migration, and angiogenesis of EPCs through PI3K/Akt signaling pathway. PLoS One 2012;7(2):e30503.

107. Wang H, Yin YG, Huang H, Zhao XH, Yu J, Wang Q, Li W, Cai KY, Ding SF. Transplantation of EPCs overexpressing PDGFR-beta promotes vascular repair in the early phase after vascular injury. BMC Cardiovasc Disord. 2016;16(1):179.

108. Lee JH, Ji ST, Kim J, Takaki S, Asahara T, Hong YJ, Kwon SM. Specific disruption of Lnk in murine endothelial progenitor cells promotes dermal wound healing via enhanced vasculogenesis, activation of myofibroblasts, and suppression of inflammatory cell recruitment. Stem Cell Res Ther. 2016;7(1):158.

109. Dai X, Yan X, Zeng J, Chen J, Wang Y, Li Y, Barati MT, Wintergerst KA, Pan K, Nyströia MA, Conklin DJ, Rokosh G, Epstein PN, Li X, Tan Y. Elevating CXCR7 improves angiogenic function of EPCs via Akt/GSK-3beta/Fyn-mediated Nrf2 activation in diabetic limb ischemia. Circ Res. 2017;120(5):e7–e23.