**Name of journal: World Journal of Stem Cells**

**ESPS Manuscript NO: 16914**

**Columns: Editorial**

**Enhancing endothelial progenitor cell for clinical use**

Ye L *et al.* Enhancing endothelial progenitor cells

Lei Ye, Kian-Keong Poh

**Lei Ye,** National Heart Research Institute Singapore, National Heart Centre Singapore, Singapore 119228, Singapore

**Kian-Keong Poh,** Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119077, Singapore

**Kian-Keong Poh,** Department of Cardiology, National University Heart Center, National University Health System, Singapore 119228, Singapore

**Author contributions:** Both authors contributed to this manuscript.

**Conflict-of-interest:** None.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to: Kian-Keong Poh, MBBChir, FRCP, FACC, Associate Professor,** Department of Cardiology, National University Heart Centre, National University Health System, 1E, Kent Ridge Road, NUHS Tower Block, Level 9, Singapore 119228, Singapore. kian\_keong\_poh@nuhs.edu.sg

**Telephone:** +65-92373289

**Fax:** +65-68722998

**Received:** February 2, 2015

**Peer-review started:** February 4, 2015

**First decision:** March 20, 2015

**Revised:** April 3, 2015

**Accepted:** May 16, 2015

**Article in press:**

**Published online:**

**Abstract**

Circulating endothelial progenitor cells (EPCs) have been demonstrated to correlate negatively with vascular endothelial dysfunction and cardiovascular risk factors. However, translation of basic research into the clinical practice has been limited by the lack of unambiguous and consistent definitions of EPCs and reduced EPC cell number and function in subjects requiring them for clinical use. This article critically reviews the definition of EPCs based on commonly used protocols, their value as a biomarker of cardiovascular risk factor in subjects with cardiovascular disease, and strategies to enhance EPCs for treatment of ischemic diseases.

**Key words:** Endothelial progenitor cells; Cell therapy; Enhancing function and number; CD34; Clinical trials

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Circulating endothelial progenitor cells (EPCs) have important potential for use in the treatment of ischemic diseases. However, their clinical application is limited bythe lack of unambiguous and consistent definitions. This article critically reviews the definition of EPCs, their status in subjects with cardiovascular disease and discusses strategies to enhance EPCs for treatment of ischemic diseases. In patients with cardiovascular conditions who may require EPC administration, EPC numbers are low and EPCs are dysfunctional. Augmenting these cells may eventually improve their clinical efficacy.

Ye L, Poh KK. Enhancing endothelial progenitor cell for clinical use. *World J Stem Cells* 2015; In press

Circulating endothelial progenitor cells (EPCs) are bone marrow derived mononuclear cells that have the capacity to migrate, proliferate, and differentiate into mature endothelial cells (ECs)[1]. Asahara *et al*[2], reported that CD34+ hematopoietic progenitor cells from adults can differentiate *ex vivo* to an endothelial phenotype, expressing endothelial cell markers and incorporating into neovessels. Since then, many publications have demonstrated the phenotype and function of these cells and use them in animal models and clinical studies[3-7].

**HETEROGENEITY OF EPC**

The translation of basic research into the clinical practice of EPCs has been limited, in part, by the lack of unambiguous and consistent definitions of EPCs. In general, two methods have been used to isolate EPCs: (1) cell selection based on surface markers and (2) cell culture and then selection. Besides CD34, a variety of cell surface markers have been shown to be associated with EPCs.

Both CD14+ monocytic and CD14- non-monocytic mononuclear cells have been used as the starting population for cultivation of EPCs[6]. Fernandez Pujol *et al*[8], demonstrated that CD14+ monocytic cells underwent a morphological transformation to oval cells and showed a clear expression of endothelial cell markers after 1 week. These markers include von Willebrand factor (vWF), VE-cadherin, CD105, acetylated low-density lipoprotein (ac-LDL)-receptor, CD36, vascular endothelial cell growth factor receptor-1 (VEGFR-1, Flt-1), and VEGF receptor-2 (KDR). However, Urbich *et al*[6], showed that CD14**-** non-monocytic mononuclear cells also expressed endothelial marker proteins and were able to form colonies. CD14**-** cells were found to incorporate into vascular structures of nude mice after hind-limb ischemia and significantly improved neovascularization.

Kalka *et al*[9], cultured human peripheral blood mononuclear cells and characterized adherent EPCs. EPCs were able to up-take ac-LDL and expressed endothelial surface markers such as von Willebrand factor (vWF), vascular endothelial growth factor (VEGF)-receptor 2 (KDR), VE-cadherin, CD146, and CD31. In a similar approach, Hill *et al*[10], found that colonial formation EPCs expressed KDR, CD31, and TIE2.

Interestingly, Hur *et al*[3], identified two types of EPCs from the same source and labelled them as early EPC and late EPC. They expressed different level of VE-cadherin, Flt-1, KDR, and CD45 markers. Late EPC produced more nitric oxide (NO), incorporated more into human umbilical vein endothelial cells (HUVEC) monolayer, and are able to better form capillary tube than early EPC[3]. However, early EPC secreted more angiogenic cytokines (VEGF and IL-8) than late EPC at culture[3]. These suggest that two types of EPC might have different roles in neovasculogenesis.

More recently, besides CD34, CD133, KDR and Ac-LDL up-take have been used more frequently as EPC markers for selection[11-14]. CD34 is a stem cell marker, CD133 an early EPC marker, KDR a marker for endothelial cells, and Ac-LDL up-take an endothelial function. Controversy remains on what are the most suitable makers for selecting EPC and what type of EPCs are most optimal for clinical application for cell therapy. Regardless of their cell surface markers, EPCs shall have the capacity to form tubular structure on Matrigel[3,4], uptake Ac-LDL[3,4], form colonies[4-6] and have the capacity to form vessels *in vitro*[2,9,15,16].

**EPC NUMBER AND FUNCTION IN CARDIOVASCULAR DISEASES**

Clinical studies documented a decreased number of circulating EPCs in coronary artery disease patients, suggesting that levels of circulating EPCs might be associated with vascular endothelial function and cardiovascular risk factors[17-21]. Hill *et al*[10] measured colony-forming units of EPCs from patients with various degrees of cardiovascular risk but without history of cardiovascular disease. A strong correlation was found between the number of circulating EPCs and the subjects’ Framingham risk factor score and between the endothelial function and the number of EPCs. It was also found that EPCs from subjects at high risk for cardiovascular events had higher rates of *in vitro* senescence than cells from subjects at low risk.

Subsequently, it was demonstrated that reduced level of circulating EPCs independently predicts atherosclerotic disease progression and development of cardiovascular events[17-19]. Sen *et al*[17], suggested that EPC determines the quality of the coronary collateral circulation. Georgescu *et al*[18] found that hypertension with hypercholesterolemia is accompanied by the alteration of vascular tone, the expression of pro-inflammatory molecules by the vessel wall, and reduced circulating EPC number.

However, contrasting results were reported, by Guven *et al*[22] and Werner *et al*[23], on the association between the level of circulating EPCs and severity of coronary artery disease. Similarly, contrasting reports were also published in patients with peripheral arterial disease (PAD)[13,24-26]. This difference might be explained by different sampling time during the studies at different part of the disease process[27] Higher circulating EPC may indicate early stage of CAD and PAD, while lower circulating EPC indicates more severe and late stage of disease, and is associated with poorer outcome of these patients. It become clear that EPC cell number and function are reduced in subjects with cardiovascular risk factors and more severe cardiovascular disease[28-32]. These are also subjects whom may benefit from EPC harvest for autologous transplantation. Thus it is important to enhance EPC cell number and function for clinical use.

**STRATEGIES TO ENHANCE EPC POTENCY**

Though animal studies of autologous EPC transplantation is feasible in both coronary and peripheral artery diseases, clinical application of these therapies are limited. Asai *et al*[33] demonstrated that topical Sonic hedgehog (Shh) gene therapy enhanced wound healing by promoting recruitment of bone marrow derived EPCs. Besides for migration, Shh enhances EPC proliferation, adhesion, and tube formation. In contrast, Jujo *et al*[34], showed that AMD3100, a CXCR4 antagonist, enhanced neovascularization and functional recovery after myocardial infarction through enhanced mobilization of EPC. Interestingly, the combinational therapy of Shh gene transfer with AMD3100 enhanced cardiac functional recovery by enhanced progenitor cell mobilization[35].

Yao *et al*[12], modified EPCs with tissue kallikrein (TK) and found that TK protected EPCs from oxidative stress-induced apoptosis via inhibition of activation of caspase-3 and -9, induction of Akt phosphorylation, and secretion of vascular endothelial growth factor[12]. Moreover, Fu *et al*[36], demonstrated that TK can enhance EPC migration and adhesion by up-regulating the expression of integrin-αβ3. Thus, TK-modified EPCs may be another strategy to enhance therapeutic potency of EPC for tissue repair.

Other agents such as statin, adiponectin, thymosin β4 (Tβ4), losartan, aliskiren,hydrogen sulfide, GTP cyclohydrolase I, and ephrin-B2/Fc have been used to enhance EPC[37-42]. Among these, statin, losartan, aliskiren, and Tβ4 have good potential to improve quality and quantity of EPCs in patients with diabetes. Statins, a commonly used class of drugs to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, have pleiotropic beneficial effect on EPC[37,43], atorvastatin increased circulating EPC after just 4 wk of administration in patients with ischemic heart failure[37]. Rousuvastatin mobilized EPC which is shown to be AKT/eNOS dependent[43].

Besides statins, losartan, an angiotensin II receptor antagonist drug, commonly used in hypertension, significantly improved impaired EPC function in hypertensive patients[42]. Tβ4, an actin sequestering protein, is found to possess angiogenic activity[44,45] and anti-diabetic property[46]. Tβ4 is angiogenic and can promote endothelial cell migration and adhesion, tubule formation, aortic ring sprouting, and angiogenesis[44,45]. Tβ4 ameliorates hyperglycemia and improves insulin resistance in mouse model of type 2 diabetes[46]. We showed that EPCs derived from Zucker DiabeticFatty (ZDF) rat have reduced angiogenic potential. Treatment with Tβ4 *in vitro* improved EPC function and survival. It also appears to have beneficial *in vivo* (unpublished results). Tβ4 appears to be a potential drug that can improve function of diabetic EPCs for cell therapy.

Clinically, EPC has been used in clinical trials[7,21,47]. In early phase clinical trials using EPCs for intramyocardial transplantation in subjects with refractory angina, Losordo *et al*[7,48] used G-CSF to mobilize bone marrow cells and selected CD34+ cells using magnetic beads post-apheresis. Subsequently these are injected into the ischemic myocardium without enhancing the cells[7,48]. Feasibility and safety appears to be established. However, efficacy remains to be demonstrated in sizeable clinical trials, preferably in a randomized and blinded manner. As subjects who need EPC therapy has lower EPC numbers and more dysfunctional EPC, these cells may need to be augmented prior to autologous transplantation. There are already several agents discussed above, which can enhance mobilization (*e.g*., AMD3100) and function of these cells. For the latter, post-processing after harvesting, may be employed. However, it will be important to make sure the cells improve and no adverse effect on these cells as a result. *In vitro* assays and animal experiments appear to show enhancement. Whether these strategies can be translated into clinical use remain to be demonstrated. It is also important to define relevant clinical end-points and surrogate parameters. Beyond statistical significance in improving markers, these need to be clinically significance. Thus, EPC migration may increase by a few mm *in vitro* in a dish or the left ventricular longitudinal strain may increase by couple of percent, it is important to show clinical relevance in translation. Besides autologous strategies, allogeneic transplantation, which has been used in animal models, may also be considered for clinical use[45,49].

**REFERENCES**

1 **Luttun A**, Carmeliet G, Carmeliet P. Vascular progenitors: from biology to treatment. *Trends Cardiovasc Med* 2002; **12**: 88-96 [PMID: 11852257 DOI: 10.1016/S1050-1738(01)00152-9]

2 **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**: 964-967 [PMID: 9020076 DOI: 10.1126/science]

3 **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**: 288-293 [PMID: 14699017 DOI: 10.1161/01.ATV.0000114236.77009.06]

4 **Ingram DA**, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 2004; **104**: 2752-2760 [PMID: 15226175 DOI: 10.1182/blood-2004-04-1396]

5 **Yoder MC**, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2007; **109**: 1801-1809 [PMID: 17053059 DOI: 10.1182/blood-2006-08-043471]

6 **Urbich C**, Heeschen C, Aicher A, Dernbach E, Zeiher AM, Dimmeler S. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation* 2003; **108**: 2511-2516 [PMID: 14581410 DOI: 10.1161/01.CIR.0000096483]

7 **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, Henry TD. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation* 2007; **115**: 3165-3172 [PMID: 17562958 DOI: 10.1161/CIRCULATIONAHA.106.687376]

8 **Fernandez Pujol B**, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML, Adamkiewicz J, Elsässer HP, Müller R, Havemann K. Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation* 2000; **65**: 287-300 [PMID: 10929208 DOI: 10.1046/j.1432-0436.2000.6550287.x]

9 **Kalka C**, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, Asahara T. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci USA* 2000; **97**: 3422-3427 [PMID: 10725398 DOI: 10.1073/pnas.97.7.3422]

10 **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**: 593-600 [PMID: 12584367 DOI: 10.1056/NEJMoa022287]

11 **Hager G**, Holnthoner W, Wolbank S, Husa AM, Godthardt K, Redl H, Gabriel C. Three specific antigens to isolate endothelial progenitor cells from human liposuction material. *Cytotherapy* 2013; **15**: 1426-1435 [PMID: 24094492 DOI: 10.1016/j.jcyt.2013.06.018]

12 **Yao Y**, Sheng Z, Li Y, Fu C, Ma G, Liu N, Chao J, Chao L. Tissue kallikrein-modified human endothelial progenitor cell implantation improves cardiac function via enhanced activation of akt and increased angiogenesis. *Lab Invest* 2013; **93**: 577-591 [PMID: 23508045 DOI: 10.1038/labinvest.2013.48]

13 **Morishita T**, Uzui H, Nakano A, Mitsuke Y, Geshi T, Ueda T, Lee JD. Number of endothelial progenitor cells in peripheral artery disease as a marker of severity and association with pentraxin-3, malondialdehyde-modified low-density lipoprotein and membrane type-1 matrix metalloproteinase. *J Atheroscler Thromb* 2012; **19**: 149-158 [PMID: 22123215 DOI: 10.5551/jat.10074]

14 **Hernandez SL**, Gong JH, Chen L, Wu IH, Sun JK, Keenan HA, King GL. Characterization of circulating and endothelial progenitor cells in patients with extreme-duration type 1 diabetes. *Diabetes Care* 2014; **37**: 2193-2201 [PMID: 24780357 DOI: 10.2337/dc13-2547]

15 **Kocher AA**, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001; **7**: 430-436 [PMID: 11283669 DOI: 10.1038/86498]

16 **Kawamoto A**, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM, Asahara T. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 2001; **103**: 634-637 [PMID: 11156872 DOI: 10.1161/01.CIR.103.5.634]

17 **Sen T**, Aksu T. Endothelial progenitor cell and adhesion molecules determine the quality of the coronary collateral circulation/Endothelial progenitor cells (CD34+KDR+) and monocytes may provide the development of good coronary collaterals despite the vascular risk factors and extensive atherosclerosis. *Anadolu Kardiyol Derg* 2012; **12**: 447; author reply 447-448 [PMID: 22626655 DOI: 10.5152/akd.2012.134]

18 **Georgescu A**, Alexandru N, Andrei E, Titorencu I, Dragan E, Tarziu C, Ghiorghe S, Badila E, Bartos D, Popov D. Circulating microparticles and endothelial progenitor cells in atherosclerosis: pharmacological effects of irbesartan. *J Thromb Haemost* 2012; **10**: 680-691 [PMID: 22303879 DOI: 10.1111/j.1538-7836.2012.04650.x]

19 **Du F**, Zhou J, Gong R, Huang X, Pansuria M, Virtue A, Li X, Wang H, Yang XF. Endothelial progenitor cells in atherosclerosis. *Front Biosci* (Landmark Ed) 2012; **17**: 2327-2349 [PMID: 22652782 DOI: 10.2741/4055]

20 **Głowińska-Olszewska B**, Moniuszko M, Hryniewicz A, Jeznach M, Rusak M, Dąbrowska M, Łuczyński W, Bodzenta-Łukaszyk A, Bossowski A. Relationship between circulating endothelial progenitor cells and endothelial dysfunction in children with type 1 diabetes: a novel paradigm of early atherosclerosis in high-risk young patients. *Eur J Endocrinol* 2013; **168**: 153-161 [PMID: 23111589 DOI: 10.1530/EJE-12-0857]

21 **Lee PS**, Poh KK. Endothelial progenitor cells in cardiovascular diseases. *World J Stem Cells* 2014; **6**: 355-366 [PMID: 25126384 DOI: 10.4252/wjsc.v6.i3.355]

22 **Güven H**, Shepherd RM, Bach RG, Capoccia BJ, Link DC. The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. *J Am Coll Cardiol* 2006; **48**: 1579-1587 [PMID: 17045891 DOI: 10.1016/j.jacc.2006.04.101]

23 **Werner N**, Wassmann S, Ahlers P, Schiegl 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**: 565-571 [PMID: 17932708 DOI: 10.1007/s00395-007-0680-1]

24 **Herbrig K**, Haensel S, Oelschlaegel U, Pistrosch F, Foerster S, Passauer J. Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells. *Ann Rheum Dis* 2006; **65**: 157-163 [PMID: 15975971 DOI: 10.1136/ard.2005.035378]

25 **Fadini GP**, Sartore S, Albiero M, Baesso I, Murphy E, Menegolo M, Grego F, Vigili de Kreutzenberg S, Tiengo A, Agostini C, Avogaro A. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol* 2006; **26**: 2140-2146 [PMID: 16857948 DOI: 10.1161/01.ATV.0000237750.44469.88]

26 **Delva P**, De Marchi S, Prior M, Degan M, Lechi A, Trettene M, Arosio E. Endothelial progenitor cells in patients with severe peripheral arterial disease. *Endothelium* 2008; **15**: 246-253 [PMID: 19065316 DOI: 10.1080/10623320802487718]

27 **Lee LC**, Chen CS, Choong PF, Low A, Tan HC, Poh KK. Time-dependent dynamic mobilization of circulating progenitor cells during percutaneous coronary intervention in diabetics. *Int J Cardiol* 2010; **142**: 199-201 [PMID: 19157595 DOI: 10.1016/j.ijcard.2008.11.198]

28 **Cubbon RM**, Kahn MB, Wheatcroft SB. Effects of insulin resistance on endothelial progenitor cells and vascular repair. *Clin Sci* (Lond) 2009; **117**: 173-190 [PMID: 19630751 DOI: 10.1042/CS20080263]

29 **van den Oever IA**, Raterman HG, Nurmohamed MT, Simsek S. Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators Inflamm* 2010; **2010**: 792393 [PMID: 20634940 DOI: 10.1155/2010/792393]

30 **Yiu KH**, Tse HF. Specific role of impaired glucose metabolism and diabetes mellitus in endothelial progenitor cell characteristics and function. *Arterioscler Thromb Vasc Biol* 2014; **34**: 1136-1143 [PMID: 24743430 DOI: 10.1161/ATVBAHA.114.302192]

31 **Zhang J**, Zhang X, Li H, Cui X, Guan X, Tang K, Jin C, Cheng M. Hyperglycaemia exerts deleterious effects on late endothelial progenitor cell secretion actions. *Diab Vasc Dis Res* 2013; **10**: 49-56 [PMID: 22561229 DOI: 10.1177/1479164112444639]

32 **Vasa M**, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001; **89**: E1-E7 [PMID: 11440984 DOI: 10.1161/hh1301.093953]

33 **Asai J**, Takenaka H, Kusano KF, Ii M, Luedemann C, Curry C, Eaton E, Iwakura A, Tsutsumi Y, Hamada H, Kishimoto S, Thorne T, Kishore R, Losordo DW. Topical sonic hedgehog gene therapy accelerates wound healing in diabetes by enhancing endothelial progenitor cell-mediated microvascular remodeling. *Circulation* 2006; **113**: 2413-2424 [PMID: 16702471 DOI: 10.1161/CIRCULATIONAHA.105.603167]

34 **Jujo K**, Hamada H, Iwakura A, Thorne T, Sekiguchi H, Clarke T, Ito A, Misener S, Tanaka T, Klyachko E, Kobayashi K, Tongers J, Roncalli J, Tsurumi Y, Hagiwara N, Losordo DW. CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. *Proc Natl Acad Sci USA* 2010; **107**: 11008-11013 [PMID: 20534467 DOI: 10.1073/pnas.0914248107]

35 **Roncalli J**, Renault MA, Tongers J, Misener S, Thorne T, Kamide C, Jujo K, Tanaka T, Ii M, Klyachko E, Losordo DW. Sonic hedgehog-induced functional recovery after myocardial infarction is enhanced by AMD3100-mediated progenitor-cell mobilization. *J Am Coll Cardiol* 2011; **57**: 2444-2452 [PMID: 21658566 DOI: 10.1016/j.jacc.2010.11.069]

36 **Fu SS**, Li FJ, Wang YY, You AB, Qie YL, Meng X, Li JR, Li BC, Zhang Y, Da Li Q. Kallikrein gene-modified EPCs induce angiogenesis in rats with ischemic hindlimb and correlate with integrin αvβ3 expression. *PLoS One* 2013; **8**: e73035 [PMID: 24019890 DOI: 10.1371/journal.pone.0073035]

37 **Oikonomou E**, Siasos G, Zaromitidou M, Hatzis G, Mourouzis K, Chrysohoou C, Zisimos K, Mazaris S, Tourikis P, Athanasiou D, Stefanadis C, Papavassiliou AG, Tousoulis D. Atorvastatin treatment improves endothelial function through endothelial progenitor cells mobilization in ischemic heart failure patients. *Atherosclerosis* 2015; **238**: 159-164 [PMID: 25525743 DOI: 10.1016/j.atherosclerosis.2014.12.014]

38 **Tie L**, Chen LY, Chen DD, Xie HH, Channon KM, Chen AF. GTP cyclohydrolase I prevents diabetic-impaired endothelial progenitor cells and wound healing by suppressing oxidative stress/thrombospondin-1. *Am J Physiol Endocrinol Metab* 2014; **306**: E1120-E1131 [PMID: 24644242 DOI: 10.1152/ajpendo.00696.2013]

39 **Liu F**, Chen DD, Sun X, Xie HH, Yuan H, Jia W, Chen AF. Hydrogen sulfide improves wound healing via restoration of endothelial progenitor cell functions and activation of angiopoietin-1 in type 2 diabetes. *Diabetes* 2014; **63**: 1763-1778 [PMID: 24487028 DOI: 10.2337/db13-0483]

40 **Broquères-You D**, Leré-Déan C, Merkulova-Rainon T, Mantsounga CS, Allanic D, Hainaud P, Contrères JO, Wang Y, Vilar J, Virally M, Mourad JJ, Guillausseau PJ, Silvestre JS, Lévy BI. Ephrin-B2-activated peripheral blood mononuclear cells from diabetic patients restore diabetes-induced impairment of postischemic neovascularization. *Diabetes* 2012; **61**: 2621-2632 [PMID: 22596048 DOI: 10.2337/db11-1768]

41 **Leicht SF**, Schwarz TM, Hermann PC, Seissler J, Aicher A, Heeschen C. Adiponectin pretreatment counteracts the detrimental effect of a diabetic environment on endothelial progenitors. *Diabetes* 2011; **60**: 652-661 [PMID: 21270275 DOI: 10.2337/db10-0240]

42 **Suzuki R**, Fukuda N, Katakawa M, Tsunemi A, Tahira Y, Matsumoto T, Ueno T, Soma M. Effects of an angiotensin II receptor blocker on the impaired function of endothelial progenitor cells in patients with essential hypertension. *Am J Hypertens* 2014; **27**: 695-701 [PMID: 24200748 DOI: 10.1093/ajh/hpt208]

43 **Zhou J**, Cheng M, Liao YH, Hu Y, Wu M, Wang Q, Qin B, Wang H, Zhu Y, Gao XM, Goukassian D, Zhao TC, Tang YL, Kishore R, Qin G. Rosuvastatin enhances angiogenesis via eNOS-dependent mobilization of endothelial progenitor cells. *PLoS One* 2013; **8**: e63126 [PMID: 23704894 DOI: 10.1371/journal.pone.0063126]

44 **Philp D**, Huff T, Gho YS, Hannappel E, Kleinman HK. The actin binding site on thymosin beta4 promotes angiogenesis. *FASEB J* 2003; **17**: 2103-2105 [PMID: 14500546 DOI: 10.1096/fj.03-0121fje]

45 **Ye L**, Zhang P, Duval S, Su L, Xiong Q, Zhang J. Thymosin β4 increases the potency of transplanted mesenchymal stem cells for myocardial repair. *Circulation* 2013; **128**: S32-S41 [PMID: 24030419 DOI: 10.1161/CIRCULATIONAHA.112.000025]

46 **Zhu J**, Su LP, Ye L, Lee KO, Ma JH. Thymosin beta 4 ameliorates hyperglycemia and improves insulin resistance of KK Cg-Ay/J mouse. *Diabetes Res Clin Pract* 2012; **96**: 53-59 [PMID: 22217673 DOI: 10.1016/j.diabres.2011.12.009]

47 **Jujo K**, Ii M, Losordo DW. Endothelial progenitor cells in neovascularization of infarcted myocardium. *J Mol Cell Cardiol* 2008; **45**: 530-544 [PMID: 18755197 DOI: 10.1016/j.yjmcc.2008.08.003]

48 **Losordo DW**, Henry TD, Davidson C, Sup Lee J, Costa MA, Bass T, Mendelsohn F, Fortuin FD, Pepine CJ, Traverse JH, Amrani D, Ewenstein BM, Riedel N, Story K, Barker K, Povsic TJ, Harrington RA, Schatz RA. Intramyocardial, autologous CD34+ cell therapy for refractory angina. *Circ Res* 2011; **109**: 428-436 [PMID: 21737787 DOI: 10.1161/CIRCRESAHA.111.245993]

49 **Poh KK**, Sperry E, Young RG, Freyman T, Barringhaus KG, Thompson CA. Repeated direct endomyocardial transplantation of allogeneic mesenchymal stem cells: safety of a high dose, "off-the-shelf", cellular cardiomyoplasty strategy. *Int J Cardiol* 2007; **117**: 360-364 [PMID: 16889857 DOI: 10.1016/j.ijcard.2006.04.092]

**P-Reviewer:** de Mello RA, Su H **S-Editor:** Ji FF **L-Editor: E-Editor:**