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HUMAN ENDOTHELIAL PROGENITOR CELL: BIOLOGICAL CHARACTERISTICS AND CLINICAL APPLICATION

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Abstract

Endothelial progenitor cells (EPCs) are cell derived from bone marrow and circulate into the peripheral circulation, they are a population of adult stem cells. These cells have characteristics similar to stem cells, but their ability to proliferate and differentiate is more limited. EPC discovery has changed the old paradigm in the field of vascular biology and it brings huge implications in medicine as EPCs can mediate the processes of vasculogenesis and maintain the vascular integrity.

In 1997, Asahara et al, has successfully isolated EPC from peripheral blood for the first time. The cells have positive surface antigen for mucosialin (CD34) and vascular endothelial growth factor receptor-2 (VEGFR-2/FLK-1). In vitro, the cells have a potency to develop into mature endothelial cells and in vivo they have roles in neoangiogenesis. Shi et al, have demonstrated that mononuclear cells which contain bone-marrow-derived-CD34+ surface antigen can be mobilized to peripheral circulation and can differentiate into mature endothelial cells. Since the discovery, mononuclear cells of peripheral blood which have CD34+ surface antigen are believed to be derived from the bone marrow and the cells are originated from the same precursor of hematopoetic stem cells (hemangioblast) since both of them show the same surface antigen, i.e. which is positive to CD34, prominin-1 (CD133) and FLK-1.

The EPC discovery has changed the old paradigm in the field of vascular biology, which believes that the process of vasculogenesis exclusively occurs only during embryogenesis. Some evidences indicate that postnatal neovascularization is not only derived from proliferation, migration and remodeling of endothelial cells on vascular wall (angiogenesis), but it also involve EPC recruitment from bone marrow, a process that has been known as vasculogenesis. The discovery has brought huge implications in medicine as EPCs can mediate the processes of vasculogenesis and maintain the vascular integrity.

Endothelial progenitor cells brings huge implications in the medical field as an EPC can mediate processes vaskulogenesis and maintain the integrity of blood vessels. Thus, EPC could potentially be used for treatment of desease caused by endothelial dysfunction and other ischemic disease. Increasing the amount of EPC in the circulation is important because it positively correlates with reendotelialisasi and neovascularization.

I. *Introduction*

Endothelial progenitor cells (EPCs) are cell derived from bone marrow and circulate into the peripheral circulation, they are a population of adult stem cells. These cells have characteristics similar to stem cells, but their ability to proliferate and differentiate is more limited. EPC discovery has changed the old paradigm in the field of vascular biology and it brings huge implications in medicine as EPCs can mediate the processes of vasculogenesis and maintain the vascular integrity.[1]

In 1997, Asahara et al[2] has successfully isolated EPC from peripheral blood for the first time. The cells have positive surface antigen for mucosialin (CD34) and vascular endothelial growth factor receptor-2 (VEGFR-2/FLK-1). In vitro, the cells have a potency to develop into mature endothelial cells and in vivo they have roles in neoangiogenesis. Shi et al[3] have demonstrated that mononuclear cells which contain bone-marrow-derived-CD34+ surface antigen can be mobilized to peripheral circulation and can differentiate into mature endothelial cells. Since the discovery, mononuclear cells of peripheral blood which have CD34+ surface antigen are believed to be derived from the bone marrow and the cells are originated from the same precursor of hematopoetic stem cells (hemangioblast) since both of them show the same surface antigen, i.e. which is positive to CD34, prominin-1 (CD133) and FLK-1.

The EPC discovery has changed the old paradigm in the field of vascular biology, which believes that the process of vasculogenesis exclusively occurs only during embryogenesis. Some evidences indicate that postnatal neovascularization is not only derived from proliferation, migration and remodeling of endothelial cells on vascular wall (angiogenesis), but it also involve EPC recruitment from bone marrow, a process that has been known as vasculogenesis.[4-6] The discovery has brought huge implications in medicine as EPCs can mediate the processes of vasculogenesis and maintain the vascular integrity.[2]

Endothelial progenitor cells (EPCs) are promising for cancer therapy because they specifically target tumors. They have the capacity to home to, invade, migrate within and incorporate into tumor structures. They are easily expanded and can be armed with therapeutic payloads protected within the progenitor cells. Once in the tumor, armed EPCs can be triggered to induce cell death in surrounding tumor cells

while being transiently protected from premature demise. In preclinical studies, therapeutic EPCs attenuated tumor growth and increased survival. Enhancing homing, self-protection and collateral tumor cell damage will increase the efficacy of EPCs for cancer gene therapy.[7]

II. *Source, Mobilization, Differentiation and Homing of EPCs*

The main source of EPCs is bone marrow, but the cells can also be isolated from peripheral and umbilical blood. Investigators have isolated or generated EPCs from many sources: mouse embryos, mouse or human embryonic stem cells, fetal liver, human umbilical cord blood, postnatal bone marrow and peripheral blood.[7] The advantage of embryonic EPCs is their unlimited proliferative capacity and the ease of genetic manipulation. These cells have the potential for systemic cancer gene therapy, as shown in a proof-of-principle study.[8] However, mature embryonic stem cell derivatives are immunogenic and ethical considerations may limit their generation. Thus, additional sources of EPCs have been explored.

In normal condition, the number of EPCs from those various sources is very limited; while the mobilization from bone marrow and the number in the circulation are extremely affected by endogenous, exogenous factors as well as physiological and pathological conditions.[9-11] The release of EPCs from bone marrow is affected by various growth factors, enzymes, ligands and surface receptors. The natural response of body tissues when there is hypoxia is increasing the production and secretion of factors that stimulate neovascularization in order to reduce hypoxia. In hypoxia state, the hypoxia-inducible transcription factor- 1α (HIF-1α) induces transcription of various proangiogenic proteins such as VEGF, stromal cell-derived factor-1 (SDF-1) and monocytes chemotatic protein-1 (MCP-1), which will actively recruit EPCs from bone marrow to the circulation and then guide them to hypoxic site. Moreover, the local condition of bone marrow has important role in EPC mobilization. The cytokines of Granulocyte colony-stimulating factor (G-CSF), matrix metalloproteinases-9 (MMP-9), VEGF, SDF-1, endothelial nitric oxide synthases (eNOS) and Nitric oxide (NO) induce mobilization by interfering the interaction between EPC and the stroma cells of bone marrow, which allow EPCs to be released from bone marrow through endothelial

sinusoid and entering the blood circulation. The process is the initial phase of EPCs mobilization from the bone marrow.[12,13]

Morphologically, EPCs cannot be identified, but there are some specific markers that can be used. In the early phase, the cells showed positive response to CD133, CD34 and VEGFR-2. Cells that shows positive response to those three markers are mainly still in the bone marrow; while the cells which are already present in peripheral circulation show diminishing expression of CD133; however, the expressions of CD34 and VEGFR-2 are still present. The next development is the advance phase of EPC (the mature endothelial cells), in which the expression of CD34 has diminished, but the VEGFR-2 is still positive and the expressions of vascular endothelial cadherin (VE-cad), platelet endothelial cell adhesion molecule-1 (PECAM-1/ CD31) and von Willebrand factor (vWF) is initiated. Markers of EPCs mobilization include SDF-1, MMP-9, GCS-F and eNOS. Marker which shows the quantity of EPCs is the expression of total CD34; while the marker for EPCs quality are viable CD34, VEGFR-2 and EPC culture cells.[13-15]

III. *EPCs in Physiological Vasculogenesis and Angiogenesis*

During development, the vasculature is formed by vasculogenesis. In this process, endothelial progenitors differentiate to endothelial cells (ECs) that form a primary capillary network. Developmental vasculogenesis depends on endothelial progenitor cells (EPCs) that are derived from a common precursor of both the hematopoietic system and the vascular system called the hemangioblast. Vascular endothelial growth factor (VEGF) and the receptor VEGFR2 are pivotal for embryonic vasculogenesis as shown by the phenotype of both VEGFR2 and VEGF knockout mice, who die in utero owing to lack of endothelial and blood cells.[7] In the adult, new vessels are built solely in healing wounds, the cycling endometrium and in growing tumors. Postnatal vessel formation was thought to proceed exclusively by sprouting from existing vessels (angiogenesis). However, it was shown that EPCs from the bone marrow participate in normal and pathological vessel formation in the adult (vasculogenesis).[4,5] Adult EPCs proliferate rapidly and share other characteristics of embryonic angioblasts. They are recruited from the bone marrow by stimuli emanating from angiogenic sites. At these sites, the EPCs differentiate into mature ECs that to some degree

incorporate into vessels. Thus, vasculogenesis appears not to be restricted to embryogenesis but to be operative in adults also. Endothelial progenitor cells from the bone marrow participate in normal and pathological vessel formation in the adult (vasculogenesis). Angiogenesis and vasculogenesis may be complementary mechanisms for vessel formation in the adult. [16]

IV. ENDOTHELIAL DYSFUNCTION AND THE ROLE OF EPC

Endothelial cell is a layer of cell covering the inner side of vascular wall. The cell responds to each physical or chemical stimulation by releasing appropriate substances to maintain vasomotor balance and vascular hemostasis. A condition, in which the endothelial cell loses its ability to maintain the balance and when the endothelial cells have lost its physiological capacity to promote vasodilation, fibrinolysis and antiagregation effect, is called as endothelial dysfunction. The pathophysiology of endothelial dysfunction is very complex and involves various mechanism through oxidative stress, i.e. a condition with increased reactive oxygen species (ROS) production and reduced antioxidant level. Increased ROS causes deprivation in NO availability, i.e. a substance produced by endothelial cells through eNOS activation.[16] Nitric oxide not only has a role in EPCs mobilization, but it also has a role in EPCs migration and proliferation. Moreover, increased oxidative stress will initiate NF-κB that may lead to inflammatory process as a beginning of atherosclerosis process. There is a dynamic correlation between inflammation, oxidative stress and EPCs mobilization. Oxidative stress stimulates inflammatory process, in which proinflammatory cytokines will stimulate the production of growth factors such as VEGF, that will further stimulate EPCs mobilization to the circulation. Inflammation may have good and bad impacts on EPCs. The limited and temporary inflammatory response can stimulate EPCs mobilization; while excessive and continuous response will cause reduced EPCs in the circulation. An in vitro study has demonstrated that inflammation and oxidative stress may affect EPC mobilization. The presence of continuous factors causes damages or continuous endothelial dysfunction which may ultimately cause reduction or exhaustion in EPCs supply. The number of EPCs in the circulation has negative correlation with the degree of endothelial dysfunction in patients with various cardiovascular risk factors.

There is a correlation between the number of EPCs and endothelial dysfunction, i.e. reduced number of EPCs can predict the incidence of cardiovascular disease in the future. The role of EPCs in vascular repair is through normalization of endothelial function and improvement of blood flow in injured vascular area. Since endothelial cells are mature cells which has poor capacity to proliferate, i.e. only about 0.01%,20 therefore, EPCs play an important role in maintaining endothelial layer through reendothelialization and neovascularization. It also indicates that EPCs can be used as a potential therapy for treating endothelial dysfunction.

V. CONDITION AND FACTORS AFFECTING THE NUMBER AND FUNCTION OF EPCS

The number of EPCs in the circulation of healthy individual is very limited, i.e. less than 1% of total bone marrow cells and less than 0.01% of total mononuclear cells in peripheral blood; however various factors and conditions can affect its number and function.[6] Evidences of some studies show that patients with cardiovascular risk factors such as age, sex, smoking habits, hypertension, diabetes mellitus (DM) and dyslipidemia have reduced number and function of EPCs. In contrast, some cytokines, hormones, medicines and physical activity can improve its number and function. A study has demonstrated that the number of EPCs is increased significantly for a short of time after acute myocardium infarction (AMI) attack and it reaches peak level on the seventh day after the attack and subsequently, the level decreases. Another study that evaluated the number of CD34+ cells in patients with heart failure shows that there is increasing biphasic response, i.e. increased CD34+ cells in patients with Class I New York Heart Association (NYHA), but it is reduced in patients with Class IV NYHA and the number is even lower when compared with control. There are physiological conditions affect the number and function of EPCs, which include different age, sex and physical activity. Estrogen has been known as a rapid stimulator for endothelial NO production and eNOS activation. In addition, estrogen can also lower the level of endogenous asymmetric dimethylarginin (ADMA), which is the inhibitor of eNOS. Fandini et al25 have reported that the number of EPC is greater in fertile female than male subjects; however, the difference has not been found in postmenopausal women

compared to men of the same age. The difference can represent the risk of cardiovascular risk in men and postmenopausal women, in which it is correlated to the low number of EPCs. In aging process, there is also an imbalance between production of free radicals and the availability of antioxidant. There is a correlation between ROS, inflammation and age, i.e. increased ROS level will potentially stimulate chronic inflammation that ultimately will cause impaired EPCs mobilization. Thus, it has also been reported that there is reduced response of EPC migration to VEGF in elderly age as well as reduced clonogenic capacity of EPC that has begun starting from the middle age. Heiss et al27 have reported that the survival, migration and proliferation capacity of the EPCs are lower in those with older age. The findings show that there is a negative effect of age on EPC differentiation and proliferation, which is correlated to cardiovascular risk. Physical exercise causes increased NO production. In a study that evaluate the effect of physical exercise on EPC in patients with stable coronary artery disease (CAD), it found that the number of EPC in the circulation increases significantly and the apoptosis is reduced after physical exercise for 28 days compared to the first day of exercise. Increased EPC number is correlated to higher NO availability after physical exercise. The study demonstrates that physical exercise increases the number of EPC in bone marrow and peripheral blood, in which the upregulation of EPC in physical exercise depends on NO and VEGF.[28] Smoking is one of risk factors causing endothelial dysfunction. The high level of oxidative stress in smoker can potentially affect EPC mobilization and survival in vivo, in which there is 50% reduction of EPC number in smokers compared to the control. Hypertension is also characterized by endothelial dysfunction and reduced NO availability. Endothelial dysfunction in patients with early stage of hypertension can induce the development of EPCs mobilization factor as the mechanism of compensation in the body. In the advanced stage of hypertension, which has continuous process of oxidative stress and inflammation, it will give contrary effect, i.e. reduced quality and quantity of EPCs.[2,13] In patients with hypertension there is accelerated aging of EPCs and reduced activity of telomerase that affect vascular remodeling. Dysfunction of EPCs in hypertension causes endothelial repair and neoangiogenesis cannot take place and it will worsen the microvascular abnormality and atherosclerosis, which is the beginning of target organ damage in

hypertension. In a study of subjects with refractory hypertension, the number of EPCs was reduced to 76.7% compared to the control. Angiotensin II accelerates the onset of EPCs aging through increased oxidative stress. A clinical trial in CAD patients has demonstrated that ramipril can increase the number of EPCs 2-5 folds as well as its function. Treatment using angiotensin II receptor antagonist also increases the number of EPCs significantly, in which olmesartan treatment can cause increased EPCs from 231+24 to 465+71; while irbesartan treatment increases the number from 196+15 to 300+23 after 4-week treatment.35 In patients with type 1 and type 2 DM, there is reduced number and function of EPC in circulation. Tapper et al36 have demonstrated that isolated EPCs from peripheral blood of patients with type 2 DM have reduced proliferation capacity in the culture up to 48% compared to normal individuals. Loomans et al37 also have reported similar results in patients with type 1 DM, i.e. there is reduced EPCs number of 44% compared to the non-diabetics control group. There is a significant increase of EPCs number in DM patients who were treated with insulin or oral diabetic agents. Insulin stimulates NO production; while in insulin resistance, there is an imbalance between endothelial damage and the ability of repair. This condition is correlated to reduced availability of NO, increased ROS production and PI3K/Akt down-regulation. It is potentially disrupt the process of vascular repair, which is the role of EPCs, including impaired EPC mobilization from the bone marrow. Increased plasma cholesterol level has significant correlation with endothelial damage and dysfunction. It has been proven that the number of EPCs is reduced significantly in patients with hypercholesterolemia and the number of EPCs has negative correlation with the level of total cholesterol and low density lipoprotein (LDL) cholesterol. Anti-inflammatory agents and antioxidants such as HMG-CoA reductase inibitors(statin) have been proven to have positive effects on EPC. Statin increases mobilization and function of EPC through upregulation of PI3K/ Akt. The effect of statin in mobilizing EPC to the circulation is correlated with increased reendothelialization and reduced neointima resulting in reduced restenosis.

VI. *EPCs in tumor neovascularization and target for tumor therapy*

The formation of new vessels within a tumor have two possible sources of endothelial cells are; migration and co-option of pre-existing vascular walls endothelial cells or recruitment of EPCs from the bone marrow. Once EPCs arrive at the site of tumor mass, they can take part in neovascularization in three ways: (1) EPCs are directly incorporated in new vessels, (2) EPCs differentiate in mature ECs, (3) EPCs produce and secrete proangiogenic factor and cytokines with paracrine effects. In cancer patients, the number of circulating EPCs is increased. [17-19..]

The need of endothelial progenitors for tumor vasculogenesis is the fundamental reason why these cells are being considered for therapeutic targeting of the tumor vasculature. EPC specifically target tumors because they have the capacity to home to, invade, migrate within and incorporate into tumor structures. EPCs can be easily expanded and manipulated without showing problems of immunological intolerance and they are easily expanded and can be armed with therapeutic payloads protected within the progenitor cell Armed EPCs can be triggered to induce cell death in surrounding tumor cells while being transiently protected from premature demise. Therapeutic EPCs attenuated tumor growth and increased survival. Enhancing homing, selfprotection and collateral tumor cell damage will increase the efficacy of EPCs for cancer gene therapy.[7]

Strategies by targeting tumour-associated EPCs:[19]

- Blocking EPC mobilisation inhibits vasculogenesis and impairs the formation of macro-metastasis in vivo
- Blocking genes involved in the homing of EPCs to tumour vasculature may carry the potential for improving antiangiogenic and antitumor effects. Another anti-tumour strategy could be to arrest the mobilisation of EPCs from the bone marrow by inhibiting some of the factors involved in their recruitment.
- Transplantation of genetically modified bone marrow progenitors may represent a vehicle for the transport of cytotoxic genes
- EPCs can be genetically engineered ex vivo by transduction with retrovirus and lentivirus vectors, which allow long-term transgene expression

VII. **Summary**

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References

1. Minhajat R, Nilasari D, Bakri S. The Role of Endothelial Progenitor Cell in Cardiovascular Disease Risk Factors. Acta Med Indones. 2015;47(4):340-7
2. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275:964-7.
3. Shi Q, Rafii S, Wu MH, et al. Evidence for circulating bone marrow-derived endothelial cells. Blood. 1998;92:362-7.
4. Smadja DM, Cornet A, Emmerich J, et al. Endothelial progenitor cells: characterization, in vitro expansion, and prospects for autologous cell therapy. Cell Biol Toxicol. 2007;23:223–39.
5. Zampetaki A, Kirton PK, Xu Q. Vascular repair by endothelial progenitor cells. Cardiovasc Res. 2008;78:413-21.
6. Eguchi M, Masuda H, Asahara T. Endothelial progenitor cells for postnatal vasculogenesis. Clin Exp Nephrol. 2007;11:18-25.
7. Debatin K-M, Wei J and Beltinger C. Endothelial progenitor cells for cancer gene therapy.Gene Ther 2008;15:780–786
8. Wei J, Blum S, Unger M, Jarmy G, Lamparter M, Geishauser A et al. Embryonic endothelial progenitor cells armed with a suicide gene target hypoxic lung metastases after intravenous delivery. Cancer Cell 2004; 5: 477–488.
9. Smadja DM, Cornet A, Emmerich J, et al. Endothelial progenitor cells: characterization, in vitro expansion, and prospects for autologous cell therapy. Cell Biol Toxicol. 2007;23:223–39.
10. Gross P, Herbrig K. Role of endothelial progenitor cells in cardiovascular. Pathology Rocz Akad Med Bialymst. 2004;49:174-7.
11. Hristov M, Weber PC. Endothelial progenitor cells: characterization, pathophysiology, and possible clinical relevance. J Cell Mol Med. 2004;8:498-508.
12. Krenning G, van Luyn MJ, Harmsen MC. Endothelial progenitor cell-based neovascularization: implications for therapy. Trends Mol Med. 2009;15(4):180-
13. Tousoulis D, Andreou I, Antoniades C, et al. Role of inflammation and oxidative stress in endothelial progenitor cells function and mobilization: therapeutic implications for cardiovascular disease. Atherosclerosis. 2008;201:236-47.
14. Esper RJ, Nordaby RA, Vilarino LO, et al. Endothelial dysfunction: a comprehensive appraisal. Cardiovasc Diabetol. 2006;23(5):1-18.
15. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol. 2004;15:83-92.
16. Marçola M, Rodrigues CE. Endothelial Progenitor Cells in Tumor Angiogenesis: Another Brick in the Wall. Stem Cells Int. 2015; 2015: 832649.
17. Botelho MC, Helena Alves H. Endothelial Progenitor Cells in Breast Cancer. Int J Immunother Cancer Res. 2016; 2: 1–2
18. Yajuan Su, Lei Zheng, Qian Wang, et al. Quantity and clinical relevance of circulating endothelial progenitor cells in human ovarian cancer. J Exp Clin Cancer Res. 2010; 29(1): 27.
19. Flamini V, Jiang WG, Lane J, et al. Significance and therapeutic implications of endothelial progenitor cells in angiogenic-mediated tumour metastasis. Crit Rev Oncol Hematol. 2016 Apr;100:177-89