Proceeding:

The 1st Makassar International Conference on Stem Cells and Regenerative Medicine.

Title:

The Role of Endothelial Progenitor Cells (EPCs) in Tumor Progression; A New Potential Therapeutic Target in Malignancies

Rahmawati Minhajat, MD. PhD. SpPD.

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. *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

V. Summary

EPCs from the bone marrow participate in normal and pathological vessel formation in the adult (vasculogenesis). 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·

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