Protein-based virtual screening: SIRT-1 as modulator maturation of circulating endothelial progenitor cells

T A Wihastuti¹*, W Nurwidyaningtyas², C T Tjahjono³ and T Heriansyah⁴

¹Department of Biomedical Nursing Science, Faculty of Medicine, Brawijaya University, Malang
²STIKES Kendedes, Malang, Indonesia
³Department of Cardiology, Faculty of Medicine, Brawijaya University, Malang, Indonesia
⁴Department of Cardiology, Faculty of Medicine, Syah Kuala University, Banda Aceh, Indonesia

*Corresponding author’s e-mail: titinwihastuti@gmail.com

Abstract. Circulating endothelial progenitor cells (cEPCs) are having involvement in the replacing vascular endothelial cells damaged or detachment from the basement membrane. cEPC needs to fulfill both quantity and quality requirements in order to play this important role. After efflux from bone marrow to circulation, niche EPC necessary of maturation to reach the potential for re-endothelialization. Risk factors exposure for cardiovascular disease not only affect the quantity and quality of cEPC, but it is also to be involved in downregulation of SIRT1. SIRT1 downregulation is mostly associated with the mechanism of senescence. Objective. to predict the direction of SIRT-1 interaction with cEPC maturation that is validated with cEPC marker surface using the STRING DB method of protein interaction. Result. SIRT1 interacts with two proteins, inhibiting VEGFA and activating and inhibiting P53. P53 inhibits the expression of p16ink4a, a protein involved in cellular senescence and P53 through AKT involved in CD 309 and ITGA2B expression. Whereas there was a direct interaction of the expression of CD 309 through the VEGFA line; CD117; TIE-2; CD 144; CD62a. Conclusion. SIRT-1 is an intracellular protein that is involved in cellular anti-aging processes but is not directly involved in the expression of EPC marker surfaces.

Keywords: surface marker, cEPC, SIRT1, maturation

1. Introduction
Circulating EPC is one of haematopoietic stem cells can be isolated either from the umbilical cord or peripheral blood [1,2,3,4]. The amount in circulation is almost associated with various conditions, including exposure to risk factors for cardiovascular disease (CVD). CEPC existence in the circulation occurs due to increased plasma levels of stromal derived factor-1 (SDF-1) which was released platelets due to injury to vascular endothelium, causing efflux of EPC from the bone marrow [5].

cEPC in circulation expresses the marker CD133 or c. Kit Ligand (CD 117) are called EPC Niche. Niche EPC is a type of hematopoietic stem cells that can differentiate into various types of single nucleated cells, one of which is EPC [6], niche EPC is still unable to perform transendothelial differentiation to replace detachment endothelial cells from the basement membrane [1]. EPC niche needs to pass maturation to the late EPC phase which is characterized by changes in marker surface to...
pre-existing endothelial residents [1,7,8]. Decreased number of cEPC or loss of potential cEPC in endothelial repair due to oxidative stress or hyperglycemia will have an impact on decreasing intracellular EPC NO synthesis. In addition, EPC functional changes were also caused by premature senescence initiation which was associated with failure of cEPC maturation due to SIRT-1 depletion [11]. SIRT-1 or Sirtuin 1 is one of the 7 target protein deacetylation enzymes that have antiaging effects in various biological processes starting from genomic maintenance, metabolic regulation, tumor suppressor and many other roles [14].

Massive oxidative stress or increase in plasma glucose will cause increased acetylation of Fox O1, which is one of the deacetylation targets of SIRT-1 which is a regulator negative of angiogenesis [10] thought to be part of premature senescence modulation in cEPC. Premature senescence in cEPC is characterized by an increase in p16 cell cycle inhibitor expression and is not related to the telomere shortening mechanism [9]. Another statement calls the expression of SIRT-1 blockade also has an impact on decreasing heterochromatin expression of protein 1α (HP1α) which is an indicator of EPC differentiation into OECs [11].

To describe the function of intracellular proteins which in this case is SIRT-1 towards cEPC maturity, an understanding of specific interactions is needed as functional partnership information which is the core of cellular processing and their systematic characterization helps to provide a context in molecular system biology [13]. However, partitioning of different pathways or complexes can be somewhat arbitrary and may not do justice to the prevalence of crosstalk and dynamic variation in the interaction landscape [13]. This study discusses the predictions of interaction between SIRT-1 and EPC maturation based on the expression of each surface marker from the niche to the late EPC.

2. Method
The STRING DB method of interaction used for analysis of the direction of interaction between SIRT-1 and various markers of maturation of endothelial progenitor cells. The STRING database (http://string-db.org) aims to provide a critical assessment and integration of protein interactions, including direct (physical) as well as indirect (functional) associations. The basic interaction unit in STRING is the functional association, i.e. a specific and productive functional relationship between two proteins, likely contributing to a common biological purpose [13] The associations in STRING include direct (physical) interactions, as well as indirect (functional) interactions, as long as they are specific and biologically meaningful [12].

The prediction of the direction of SIRT interaction will be directed at several different cEPC markers including:

- CD 133 (prominin I)
- CD 117 (cKitL)
- CD34, Asahara et al, 1997
- CD 309/ KDR/ VEGFR-2
- TEK/ TIE-2
- CD31/PECAM/
- CD144 / CDH5/ VE-cadherin
- CD41 /vWf
- CD45
- E-selectin / CD62E/ SELE

3. Result
Figure 1 shows that directly SIRT-1 interacts with two proteins that inhibit the activation and inhibition VEGFA and P53. P53 inhibits the expression of p16ink4a / CDKN2a, a protein involved in cellular senescence and P53 through AKT involved in the expression CD 309 / KDR and ITGA2B. While through the VEGFA pathway, there is a direct interaction of the expression CD 309 / KDR; CD117 / KIT; TIE-2 / TEK; HBA1; CD 144 / CDH5; CD62a / SELE.
SIRT-1 has a non-specific direction with Akt1, but with p53 showing many types of interactions that can be identified include post-translational modification, activation, transcriptional regulation, conversely p53 shows the interaction of inhibition of p53 activity.

CDKN2 as a downstream effect of p53 appears that the interaction model of p53 inhibits CDKN2A expression but on the other hand CDKN2A expression is reported to activate p53. Between CDKN2A and p53 there is an interaction binding or in post translational modification with an unspecified type of effect. There is an indirect interaction between SIRT-1 and CDKN2A or p16 through p53 as in the previous publication. SIRT-1 does not directly interact with the entire eEPC marker in all phases of maturation. And SIRT-1 regulates VEGFA transcription (inhibition) and VEGFA expression directly inhibits SELE, CDH5 and TEK expression but there are publications that report VEGFA inducing CD34 and TEK expression.

Figure 1. SIRT1 interaction model with various cEPC marker surfaces.

4. Discussion
Protein interactions (PPIs) handle a wide range of biological processes, including cell-to-cell interactions and metabolic and developmental control [15]. In silico is one of three forms of method that are widely used to identify protein-protein interaction.

This method uses computer simulations to understand the total context of the interaction potential, and it is better to develop a range of possible interactions between proteins. The results of this study showed no direct interaction between SIRT1 and CD antigen expression used as a marker of cEPC maturation.

Cluster differentiation (CD) antigen is surface molecules expressed on many types of cells and designate a spliced variant of the extracellular domain [16]. The CD antigen is protocol used for identification and investigation of molecules providing targets for immunophenotyping of cells. CD antigens can act in a lot of ways, like receptors or ligands in terms of physiology. As a signal, antigens usually initiated CD, altering the behavior of the cell. Some CD proteins do not play a role in cell signaling, but have other functions, such as cell adhesion [9]. The results of this study mention that there is a direct interaction between SIRT1 and p53 but it is not an interaction with cEPC marker surface expression.
The involvement of SIRT1 depletion in premature senescence was identified through p53 acetylation activity which would later modulate promyelocytic leukemia protein (PML) - (a protein that mediates premature senescence at cellular level). The inhibition of SIRT1 expression by miR-127 causes senescence in endothelial cells, including exposure to conditions for hyperglycemia, which also have the same effect. In other words the decrease in deacetylation activity and fungi by SIRT1 will have an impact on increasing acetylation and stabilization of p53, which then mediates various senescence cellular [14].

SIRT1 is also maintenance of haematopoetic stem-cell (HSC) homeostasis so that the decrease in SIRT1 expression will increase DNA damage, age-related molecular accumulation and aging phenotype in HSC and also on endothelial progenitor cells (EPC).

Failure to repair damaged DNA causes instability of genomics and contributes to premature cellular senescence and increased aging. Under these conditions, SIRT1 induces these improvements through deacetylation of Ku70 proteins, including in cases of atherosclerosis in vascular smooth muscle cells (VSMC). SIRT1 initiates activation of Nijmegen breakage syndrome-1 repair protein (NBS1) and not p53 [14].

Another possibility is that cEPC maturation failure is associated with a decrease in the amount of cellular ATP due to increased production of mitochondria-derived reactive oxygen species (mROS) which reacts with NO to form ONOO- which inhibits PGC-1α protein synthesis by mitochondria [18]. Another controversy states that premature senescence due to a decrease in SIRT1 is not related to activation of p53 / p21 but rather the stress induced premature senescence mechanism along with the increase in p16INK4a [19].

So this study provides information that surface molecule expression is not related to intra cellular signaling by SIRT1, p53, p16 or PGC1α.

5. Conclusion
SIRT1 is an intracellular protein that is involved in the anti-aging process at cellular level. But SIRT1 is not directly involved in the expression of marker surfaces from EPC. The clusters of differentiation are a molecule in the form of a ligand or receptor that is widely used to identify cell characters based on surface markers with an immunophenotyping approach and this molecule does not always play a role in cell signaling.

References
[1] Tagawa S, Nakanishi C, Masayuki Mori, Yoshimuta T, Yoshida S, Shimojima M, Yokawa J, Kawashiri M, Yamagishi M, and Hayashi K 2015 Determination of early and late endothelial progenitor cells in peripheral circulation and their clinical association with coronary artery disease, Hindawi Publishing Corporation International Journal of Vascular Medicine Volume 2015, Article ID 674213, 7 pages
[2] Zhao J, Mitrofan CG, Appleby SL, Morrell NW, and Lever AML, Disrupted Endothelial Cell Layer and Exposed Extracellular Matrix Proteins Promote Capture of Late Outgrowth Endothelial Progenitor Cells, Stem Cells International Volume 2016, Article ID 1406304, 13 pages
[3] Zhang M, Rehman J, Malik AB, Endothelial progenitor cell and vascular repair, Curr Opin Hematol. 2014 May ; 21(3):224-228
[4] Liu W, Ren L, Wang T, Navarro N, Tang L, The Involving Roles of Intrahepatic and Extrahepatic Stem/Progenitor Cells (SPCs) to Liver Regeneration, International Journal of Biological Sciences 2016; 12(8): 954-963
[5] Pecchi VG, Sara Valdés , Véronique Pons , Paula Honorato, Laurent O. Martinez, Liliana Lamperti, Claudio Aguayo, Claudia Radojkovic; Apolipoprotein A-I enhances proliferation of human endothelial progenitor cells and promotes angiogenesis through the cell surface ATP synthase, Microvascular Research 98 (2015) 9–15
[6] Hirstov M, Erl W, Weber PC. Endothelial Progenitor Cells Mobilization, Differentiation, and
Homing, Arterioscler Thromb Vasc Biol. 2003 23 1185-1189

[7] Malinovskaya NA, Komleva YK, Salmin VV, Morgun AV, Shuvaev AN, Panina YA, Boitsova EB and Salmina AB (2016) Endothelial Progenitor Cells Physiology and Metabolic Plasticity in Brain Angiogenesis and Blood-Brain Barrier Modeling. Front. Physiol. 7 599

[8] Hur J, Yoon CH, Kim HS, Choi J, Kang H, Hwang KK, Oh B, Lee M, Park YB (2004) Characterization of Two Types of Endothelial Progenitor Cells and Their Different Contributions to Neovascuologenesis, Arterioscler Thromb Vasc Biol. 2004; 24:288-293. available at http://www.atvbaha.org

[9] CD antigens / Cluster of Differentiation. https://www.sinobiological.com/cd-antigens-cluster-of-differentiation.html.

[10] Tarantino C, Paolella G, Cozzuto L, Minopoli G, Pastore L, Parisi S, Russo T. miRNA 34a, 100, and 137 modulate differentiation of mouse embryonic stem cells. FASEB J. 2010; 24 3255–3263

[11] Berezin AE. Epigenetically Modified Endothelial Progenitor Cells in Heart Failure. J Clin Epigenet. 2016, 2:2

[12] Sklarczyk D, Morris J, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva T. Roth A, Bork P, Jensen LJ, Mering CV, The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible, Nucleic Acids Research, 2017, Vol. 45, Database issue

[13] Sklarczyk D, Franceschini A, Wyder S, Foslund K, heller D, Huerta JC, Simonovic M, Roth A, Santos A, Tsafao KP, Kuhn M, Borl P, Jensen LJ, Mering C, STRING v10: protein-protein interaction network, integrated over the tree of life, Nucleic Acids Research, 2015, Vol. 43, Database issue D447–D452

[14] Ghosh S, Zhou Z, SiRTain regulators of premature senescence and accelerated aging, Protein Cell 2015 6 (5) 322–333

[15] Srinivasa V, Srinivas K, Sunand GN, Protein-protein interaction detection : method and Analysis, International Journal of Proteomics Volume 2014, Article ID 147648, 12 pages

[16] Engel P, Boumcell L, Balderas R, Bensussan A, Gattee V, Horejsi V, Jin B, Malavasi F, Mortari F, Schwartz R, Stockinger H, Zelm MC, Zola H, Clark G, CD Nomenclature 2015: Human Leukocyte Differentiation Antigen Workshops as a Driving Force in Immunology, J Immunol 2015; 195:4555-4563

[17] Maeng YS, Young Kwon J, Kweon Kim E, Kwon YG, (2015). Heterochromatin Protein 1 Alpha (HP1α: CBX5) is a Key Regulator in Differentiation of Endothelial Progenitor Cells to Endothelial Cells: HP1α is an EPC Differentiation Inducing Factor. STEM CELLS. 33

[18] Gomes AP, Price NL, Ling AJ, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP, Mercken EM, Palmeira CM, de Cabo R, Rolo AP, Turner N, Bell EL, Sinclair DA 2013 Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell. 155(7):1624-38.

[19] Vassallo PF, Simoncini S, Ligi I, Chateau AL, Bachelier R, Robert S, Morere J, Fernandez S, Guillet B, Marcelli M, Tellier E, Pascal A, Simeoni U, Anfosso F, Magdinier F, George FD and Sabatier F, (2014) Accelerated senescence of cord blood endothelial progenitor cells in premature neonates is driven by SIRT1 decreased expression, Blood 123 2116-2126