Aldehyde-Low Endothelial Progenitor Cells in Ischemic Wound Repair, Regulated by SDF1/CXCR4

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ABSTRACT

The incidence rate of both ischemic heart disease and ischemic stroke over the past decade has become a concern in the respect of global mortality rate. Ischemia is a condition of oxygen deprivation in the specific tissue which leads to loss of organ function. Regenerative medicine by using the endothelial progenitor cells (EPCs) show a great potential, especially the Alde-Low EPCs. The Alde-Low EPCs are regulated mainly by the chemo-attractant proteins of SDF1/CXCR4 which responsible for the cell migration and is regulated by the HIF2α. Further investigation and research on the correlation of SDF1/CXCR4 and the Alde-Low EPCs are needed.

Keywords: Alde-low EPCs, CXCR4, HIF2α, SDF1.

ABSTRAK

Peningkatan kejadian dan kematian akibat penyakit jantung iskemik dan *stroke* iskemik secara global pada beberapa dasawarsa telah menjadi perhatian. Upaya regenerasi menggunakan sel progenitor endothelial (EPCs) memperlihatkan potensi sangat tinggi, khususnya Alde-rendah EPCs. Alde-rendah EPCs diregulasi oleh protein kemoatraktan dari SDF1/CXCR4 yang bertanggung jawab untuk migrasi sel dan diregulasikan oleh HIF2α. Hubungan SDF1/CXCR4 dan Alde-rendah EPCs masih perlu diteliti lebih lanjut. **Dias Rima Sutiono, Candra Louis. Peran Progenitor Sel Endothelial Rendah *Aldehyde* dalam Penyembuhan Luka Iskemik Diregulasi oleh SDF1/CXCR4**

Kata kunci: Alde-rendah EPCs, CXCR4, HIF2α, SDF1

INTRODUCTION

The advance in the field of regenerative medicine, especially on the application of endothelial progenitor cells in curing ischemic wound has shown promising results on several *in vivo* studies. Ischemic wound is defined as tissue death due to the restricted oxygen supply, creating a hypoxic condition around the localized area of specific tissue. As reported by (World Health Organization) WHO, ischemic heart disease and stroke, as one of the most common and prevalent type of ischemic wound, have become the leading cause of death with the total contribution of 13.2% and 11.9% respectively between 2000 and 2012 (WHO, 2014). The ischemic wound in circulation system usually leads to complication on the cardiac level due to the blocked or reduced oxygen supply which later also affects the oxygen supply to the cerebral area that in turn causes ischemic stroke. On the other hand, EPCs which have been found to be responsible in angiogenesis and wound healing under hypoxic condition, under the regulation of hypoxia-inducible factors (HIF).[1] Despite the potential of EPCs in curing ischemic wound, the fundamental of defining EPCs has become an unresolved issue in the respect of numerous methodologies in isolation and characterization. In this review paper we will specifically discuss on using aldehyde dehydrogenase (ALDH) activity as the basis in EPCs classification – instead of the cell receptors. The recent studies on *in vivo* study of Alde-Low EPCs, under HIF2α regulation, exhibit a great potential in curing ischemic wound will be presented.

Aldehyde Dehydrogenase as Biomarkers

One of the standards in EPCs classification relied on the cell surface receptors expressed differently by the early and late EPCs. The early EPCs exhibit the expression of $CD34^+$, $CD45^+$, and $CD14^+$, develops within the first week of culture.[2-4] The late EPCs are identified to dominate the early EPCs within the second week of culture with VEGFR-2, $CD34^+$, and $CD31^+$ with no myeloid biomarkers expressed in early EPCs.[2] Yet, the standard in the process of development and characterization of EPC has not developed in which various combinations of expressed biological markers become an issue in further EPCs classification.

On the other hand, it has been proven if the EPCs could be classified by using the expression level of aldehyde dehydrogenase. ALDH has been identified as an enzyme which is involved in the breakdown cytotoxic aldehydes, cell differentiation, and drug resistance.[5] On the same time, it has been found to be highly expressed in both cancer cells, stem cells, and progenitor cells.[6,7] The level of ALDH expressed by progenitor cells vary and is determined by using flow cytometry with dansylaminoacetaldehyde as the fluorescent protein (Jones, 1995). It was found if ALDH is negatively correlated with the HIF-1α and HIF2α in the case of breast cancer cells proliferative ability and drug resistance.[8,9] According to Nagano et al. both high and low ALDH EPCs derived from human

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umbilical cord blood (UCB) did not express the CD45 and CD14; whereas CD34, CD166, and c-kit of the endothelial cell (EC) markers were observed, with a higher concentration of CD34 cells in Alde-Low EPCs.[10]

HIF2α and SDF1/CXCR4

Hypoxia-inducible factor is a stressor expressed by the cells under hypoxic condition, inducing angiogenesis and cell migration. The HIF-α subunitis divided into three isoforms of HIF-1α, HIF2α, and HIF-3α.[11] Despite of belonging to the same family, in retinal carcinoma and endothelial cells HIF-1α was proven to be antagonistic to the HIF2α – which induce proliferation through the c-Myc. However, in EPCs, Hoenig et al. reported HIF1-α as the main regulator of neovascularization.[12] Nonetheless, Tu et al. stated HIF2α as the main regulator in ischemic wound healing through SDF1/CXCR4 pathway in Alde-Low EPCs.[13] Furthermore, it needs to be noted if Tu et al. also mentioned if both HIF-1α and HIF2α were elevated on the Alde-Low EPCs, in which there might be a correlation between HIF-1α and HIF2α in ischemic wound healing.

SDF-1, is a ligand member of CXC chemokine family with its receptor CXCR4, a GPCR, where both molecules are essential in carrying cellular response of cell migration and proliferation.[4] Mutation resulting in SDF1/CXCR4 absence or interference has been shown to cause abnormal organogenesis of cardiac and restricted hematopoiesis.[14] In tissue repair, SDF1/CXCR4 has also been found to be accumulated in acute ischemic brain and ischemic skin flap are going to be discussed further briefly.[15] The CXCR4 therapy was actually approved by the FDA and is currently going for the clinical trials specifically for the application on treating the cancer, HIV, and Whim Syndrome, as reported by Debnath et al. in their review, but there none of EPCs therapy approved.

SDF1/CXCR4 Alde-Low EPCs in vivo trials

In vivo trial condition of hypoxic condition which is defined as low oxygen availability below 1%, this is usually done by placing the subject in hypoxic container. In the recent finding by Tu et al., Alde-Low and Alde-High UCB-derived EPCs of knocked-out HIF2α and HIF1α, together with the wild type (WT) were observed in their ability of healing the induced-ischemic skin flap tissue for over seven days. The results then showed if the knocked out HIF1α mouse still has its ability in healing the ischemic area of skin-flap, while a significant reduced wound healing ability was observed in the knocked-out HIF2α mouse. Moreover, the CXCR4 expression level was also significantly reduced along with the Vascular Endothelial Growth Factor (VEGF), which shows a direct correlation between HIF2α and CXCR4. The results also conclude if the Alde-Low EPCs under various conditions still has higher ischemic wound healing ability compared to the Alde-High EPCs.[13]

While separately, the potential of SDF1/CXCR4 in the UCB-derived Alde-Low EPCs recruitment as chemo-attractant towards the chronic ischemic brain tissue has also been proven by the work of Nakamura et al. where Alde-Low and Alde-high EPCs was being injected into the mouse and later observed after 24 hours. The result shows if Alde-Low EPCs manage to migrate from the tail to the ischemic brain tissue, in which accumulated SDF1/CXCR4 was also observed. In addition, the Alde-Low EPCs exhibit significant ability in reducing the infarct area for over 24 hours compared to the Alde-High EPCs.[15]

CONCLUSION

The research on Alde-Low EPCs potential is still developing, especially on the axis of SDF1/CXCR4 and HIF2α. While further classification of EPCs is needed, the basis of using ALDH as the standard in determining EPCs should be taken into consideration, since the mortality rate caused by ischemic heart disease and stroke will increase along with the life expectancy.

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