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Article Title: The evidence of sophisticatedly heterogeneous population of human umbilical vein endothelial cells

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INTRODUCTION

Endothelial cells (ECs) is the thin layer of cells that lines the interior surface of blood vessels and lymphatic vessels and play an important role in a diverse range of physiological and pathophysiological processes, including vasculogenesis and neo-angiogenesis, vasoconstriction and vasodilation, blood clotting, inflammation, and facilitating tissue-repair and tumor growth. Human umbilical vein endothelial cells (HUVECs) are cells derived from the endothelium of veins from the umbilical cord, and HUVECs have been used as a laboratory model cells to realize the function and pathology of ECs. Recent study have reported that HUVECs can be conversed into multipotent stem-like cells to by an activin-like kinase-2 (ALK2) receptor dependent mechanism(1). Athanasia D. Panopoulos *et al* found that the rapid reprogramming of HUVECs, with an efficiency approximately 300-fold higher than human fibroblasts (2). In our previous study, we show that the specified hepatic cells could self-organize into three-dimensional iPSC-Liver-Buds by recapitulating organogenetic interactions between HUVECs and mesenchymal stem cells (MSCs) (3). Those evidences indicated that HUVECs will play a significant role in regenerative medicine, so the distinct population of HUVECs and the harbored endothelial progenitor cells (EPCs) are necessary to isolate, and characterize.

MATERIALS AND METHODS

Isolation and culturing of HUVECs

Under the permission of Ethical Review Board of our University as well as informed consent from all study subjects, freshly isolated umbilical cords were obtained from the Hospital of Yokohama City University. The surface of umbilical cords was washed and the umbilical vein was flushed by Hanks' Balanced Salt Solution (HBSS, Gibco). Then, the vein vessel was filled with 1 g/L

collagenase in RPM1640 medium, and both ends of the umbilical vein of cords were bound.

Umbilical cords were immersed into HBSS, and incubated for 15 min at 37°C. Endothelial cells were washed out with HBSS. Cell suspension was centrifuged at 200 g for 3 min. The separated cells were cultured with EGM medium (Lonza). When the cells grew over the 80% space of the dish, cells were digested with 0.05% trypsin-EDTA (Gibco). Cell suspension was centrifuged at 200 g for 3 min. The cells were inoculated into the culture dish at about 3×10^3 cell/cm² concentration, and the cell propagation and collection procedure were repeated again as described above.

Flow cytometric analysis

Harvested HUVECs were stained with anti-human monoclonal antibodies (all from BD Pharmingen) as CD13, CD31, CD34, CD45, CD104, CD117/c-kit, CD144, CD146, CD140a, CD271, and VEGF2 at 4°C for 30 min. After incubation, cells were analyzed on a Beckman Coulter MoFLO™ ASTRIOS (Brea, CA).

RESULTS

To evaluate the endothelial progenitor character, we performed the clonal colony assay in the fresh isolated HUVECs. The results from almost all of clinical specimen showed that the frequency of Endothelial Colony-Forming Unit in Culture (E-CFU-C) is high in primary HUVECs. All of the CD31-positive endothelial cells expressed CD34 with immunofluorescent staining for umbilical cord sections and with flow cytometry assay for primary cells. After culture, the rate of CD34 positive cells decreased dramatically. The antigenic profiling of early passage HUVECs then, was analyzed for the markers of progenitor cells, endothelial cells, growth factor receptors and adhesion molecules (Figure 1). 95.1% of HUVECs was positive for CD31, and 92.4% of those cells showed CD13 expression. Those cells also expressed endothelial markers VEGFR2 (41.8%),

CD144 (48.1%) and CD146 (69.5%). CD34 and c-kit also known as hematopoietic stem cell markers expressed down to 21.6% and 25.1% respectively after two-passage cultures. CD45, CD271, CD104 and CD140a were negative or low expressions. According to the expression of the surface markers CD34 and c-kit, the early passage HUVECs could be divided into different subpopulations and the distinctive character among the subpopulations could be found in the cell morphology, proliferative capability and angiogenesis.

DISCUSSION AND CONCLUSION

The application of HUVECs spreads widely. To comprehensively understand the cell characters in different subpopulations including the harbored progenitor cells will contribute much to the application such as in regenerative medicine. Antigenic profiling demonstrated that over 95% of the early passage HUVECs expressed the endothelial marker CD31, while some of them expressed the endothelial marker VEGFR2, CD144 and CD146. The varied marker expressions and the distinct proliferation capability and the angiogenesis among the different subpopulations suggested that HUVECs are comprised of heterogeneous population. It has been known that the circulating endothelial progenitor cells (EPCs) can be divided into hematopoietic lineage EPCs and non-hematopoietic lineage EPCs (4), while the HUVEC progenitor is still a question in the field. The double positive cells of CD34 and c-kit could be found in this cell pool, which the markers expressed on hematopoietic stem cells (HSC). Furthermore, CD271, the specific marker for bone marrow-derived mesenchymal stem cells (MSC) (5), was undetectable. These results suggest that endothelial progenitor cells in HUVECs should share a common origin with the HSC. Human umbilical vein endothelial cells, a widely used human cell source should be isolated and characterized accurately in the future.

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Figure Legend

Figure 1. Antigenic profiling for early passage HUVECs.

Data presented as histogram overlays and the percentage indicated the labeled marker rate. Filled histograms represent isotypic controls and open histograms are the cells labeled with various antibodies. Values on the x axis indicate fluorescence intensity expressed in log scale and the number of events is expressed on y linear scale.

Figure

Figure 1. Antigenic profiling for early passage HUVECs.