**Cells with hematopoietic activity in the mouse placenta reside in side population**

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The discovery of a major hematopoietic stem cell (HSC) pool in midgestation mouse embryo has defined the placenta as an important anatomical site that participates in HSC development. Placental hematopoietic activity culminates in a rapid expansion of the definitive HSC pool, which enables placenta to harbor a large pool of hematopoietic stem cells especially during midgestation (embryonic days (E) 12.5-E13.5). In this study, I examined the flow cytometric pattern of placenta cells, in view of CD45 (a hematopoietic lineage marker) and c-Kit (a hematopoietic stem cell marker and a receptor for stem cell factor, SCF) expression, in mice from E10.5 to E15.5. I also determined which types of these cells have the highest hematopoietic property and ability to differentiate towards multiple lineages by performing (1) co-culture with OP9 stromal cells and (2) colony forming assay in semisolid methylcellulose medium. Only $CD45^{+}c\text{-}Kit^{+}$ population of cells, when co-cultured with OP9 stromal cells, showed the property to form cobble stone colonies which are believed to have hematopoietic differentiating ability. When cultured in semisolid methyl-cellulose medium, only this population showed the ability to form colonies including multiple lineages. Moreover, the cells that have hematopoietic activity were enriched in the $CD45^{+}c\text{-}Kit^{+}$ $CD34^{+}$ fraction.

The $CD45^{+}c\text{-}Kit^{+}$ population of cells expressed high levels of CD34 (a marker of hematopoietic progenitors and endothelial cells), CD49d (integrin$\alpha$4), and CD31 (a marker of endothelial cells). In this population, expression levels of CD41 (a marker of hematopoietic progenitor and megakaryocyte marker), Sca-1 (a marker of lymphopoietic stem cell), Mac-1 (a marker of macrophage) and VE-cad (VE-cad, a marker of endothelial cells) were low. To

examine the reconstitution activity of $CD45^+c\text{-}Kit^+$ population, I infected $CD45^+c\text{-}Kit^+$ cells with EGFP-expressing retrovirus and cocultured with OP9 stromal cells. I found a large number of cobble stone colonies after the secondary and tertiary replating. These results were confirmed by performing reconstitution assays using the single cell culture of $CD45^+c\text{-}Kit^+$ cells. To assess which fraction of placenta cells (the fetal part or the maternal part) have the hematopoietic activity, transgenic GFP heterozygous mice in which the fetal part of placenta is GFP positive ($GFP^+$) and the maternal part is GFP negative ($GFP^-$) were used. E11.5 and E13.5 $CD45^+c\text{-}Kit^+$ placenta cells that have an ability to form hematopoietic colonies are the fetal $GFP^+$ placenta cells.

Hematopoietic stem cells have been reported to be enriched in the hoechst dye-effluxing "side population" (SP). I performed interesting approach by gating the SP cells in combination with the expression profile of CD45 and c-Kit. E11.5 and E13.5 $CD45^+c\text{-}Kit^+$ placenta cells that have the ability to form hematopoietic colonies mainly reside in the side population. Among the placenta from E10.5 to E15.5, the $CD45^+c\text{-}Kit^+$ cells of E12.5 have the highest ability to form hematopoietic colonies.

Taken together, in the placenta of mouse embryo, we conclude that, $CD45^+c\text{-}Kit^+$ cells from E10.5 to E15.5 have the ability to form hematopoietic colonies, and that, from the developmental viewpoint, the E12.5 $CD45^+c\text{-}Kit^+$ placenta cells have the highest ability. In addition, in the E11.5 and E13.5 only the fetal ($GFP^+$) and side population of $CD45^+c\text{-}Kit^+$ cells have the ability to form hematopoietic colonies when co-cultured with OP9 stromal cells and cultured in methylcellulose.