Supplemental Legends

Supplemental Figure 1. Flow cytometrical analysis of primitive and definitive EPC-CFUs.

After 7 days culture of BM Lin-/Sca-1+ cells in EPC-CFA, each colony cell was individually harvested as previously reported.


In brief, white spotty colonies (primitive colonies) in each dish were collected with adding cold PBS three times. Then, wells were incubated for 5 minutes at 37°C with one ml PBS/EDTA(5mmol/L), and collected adhesive definitive colonies. The suspended cells in PBS/EDTA(5mmol/L) were aliquoted in 200µl each per tube and stained for FACS, with 2µl each of anti-mouse antibodies for PE/Cy7 conjugated CD45 (Biolegends, San Diego, CA). APC conjugated Flk-1 (eBioscience). Colony cells in suspension of PBS/EDTA(5mmol/L) primarily reacted with a biotin-conjugated rat anti-mouse antibody for 11b (BD Pharmingen) or Tie-2 (eBioscience) were stained with APC-conjugated streptavidin (Biolegends). Separately, the cells were also reacted with a rabbit polyclonal antibody for von Willebrand factor (vWF) (Santa Cruz biotechnology, Inc., Santa Cruz, CA) was stained with APC-conjugated anti-rabbit antibody (Biolegends).

The values indicate the percentages of positive cells for the investigated surface antigens. The percentages of positive cells for endothelial markers of Flk-1 and vWF together with monocyte markers of CD45 and CD11b were upregulated in definitive EPC colony cells vs primitive, indicating that definitive EPC-CFU predominantly possesses monocyte/endothelial feature vs primitive. The findings present that BM derived EPCs in circulation exhibits the feature of monocyte/endothelial lineage cells, according to the differentiation.

Supplemental Figure 2. Retinal vascular features in murine OIR model after injection of colony cells of each primitive or definitive EPC-CFU.

In EPC-CFA of Lin-/Sca-1+ cells from EGFP Tg mice, primitive and definitive colony cells at day 7 culture were separately corrected as aforementioned in Supplemental Figure 1. Each colony cells (1x10^5 cells /eye) were intravitreally injected in OIR+ mice at P14 with
33G needle (Ito Corporation) under inhalation of Isoflurane (Abbott, Japan).
At P17, vascular structures of flatmounted retinas were investigated by immunochemistry for CD31 and observed as aforementioned in MATERIALS AND METHODS. Avascular and neovascular areas were measured by image software (Photoshop Elements9; Adobe system, Mountain View, CA) according to the reported procedure.


(a) Illustration of transplanted procedure of colony cells from each EPC-CFU in OIR. (b) Pathological vascular features in retina of murine OIR model after injection of each colony cell from primitive or definitive EPC-CFU (upper panels). Avascular and tufts areas in the upper panels are indicated by white and yellow colored highlights (lower panels). (c) Quantitative evaluation of pathological vascular development of avascular area (left) and neovascular area (right), n=4. Injected definitive colony cells significantly abrogated both of avascular and neovascular areas, compared to primitive. (d) GFP+ definitive colony cells were incorporated into both of intact retinal vessels and tufts as endothelial lineage cells (asterisk) but also as glial cells contacting vasculatures (arrowheads), whereas GFP+ primitive colony cells were neither incorporated into intact nor neovascularites, but existed at the outside of vascular structures (arrowheads).

Supplemental Movie.
BM-derived GFP+ cell was three dimensionally co-staining with CD31 indicating intra-vascular EPC. GFP, green; CD31, red.