

Introduction

Human induced pluripotent stem cells (iPSCs) represent promising cell resource for disease modeling, drug screening and regenerative medicine. Since iPSCs are originally derived from somatic cells, tissue-specific epigenetic memory has been observed in early passage iPSCs, which may interfere the directed differentiation towards target lineages. For example, blood-derived iPSCs have greater differentiation efficiency to hematopoietic lineage than fibroblast-derived iPSCs, whereas beta cell-derived iPSCs have increased ability to differentiate into insulin-producing cells when compared to the isogenic non-beta iPSCs or ESCs. The somatic epigenetic memory of tissue-of-origin may be somewhat diluted with extensive passaging. However, extensive culture doesn't significantly improve the epigenetic resemblance of iPSCs to authentic embryonic stem cells. Here we derived human iPSC from three types of somatic cells from the same individuals: fibroblast (FB-iPSCs), endothelial cells (EC-iPSCs) and cardiac progenitor cells (CPC-iPSCs). We then differentiated these iPSCs into endothelial cells and compared their molecular characteristics and functional behaviors in vivo and in vitro.

Methods & Materials

Donor cell isolation: human fibroblast, endothelial cells and cardiac progenitor cells were derived from the skin, aorta and heart of two aborted fetuses, respectively. The CPCs were enriched by using Sca1 coupled magnetic beads.

Human iPSC generation: human FBs, ECs and CPCs were reprogrammed by lentiviral infection with a vector carrying OCT4, SOX2, KLF4 and C-MYC transgenes.

EC differentiation: hiPSCs were first induced to embryoid bodies (EBs) by depletion of bFGF and then differentiated into EC lineage by sequential administration of Activin, BMP4, bFGF and vEGF. EBs were harvested on day 14 and sorted by FACS using CD31/CD144 monoclonal antibodies.

Hindlimb ischemia model: hiPSCs were tagged with a triple fusion construct carrying Fluc, RFP and HSVtk. One million cells were intramuscularly injected around ischemic area and monitored by optical bioluminescence.

Single-cell qPCR: single cells were captured by using C1 single cell auto-prep arrays and then single-cell qPCRs were conducted on Biomark 48.48 Dynamic Array chips using the Biomark HD system (Fluidigm).

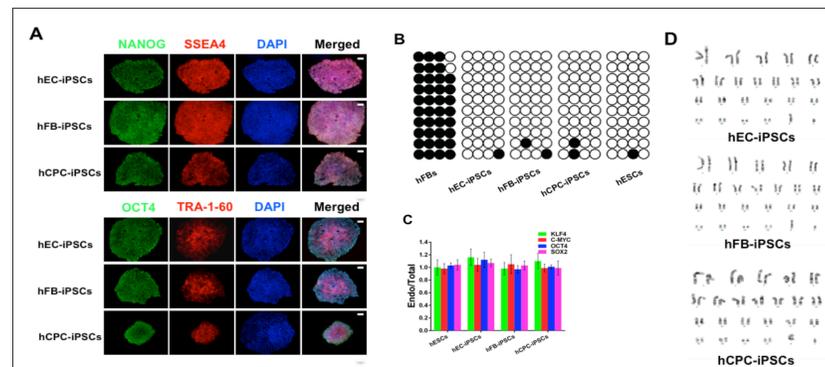


Figure 1 Human iPSCs generation and characterization. These iPSC lines were positive for ESC-specific markers TRA-1-60, SSEA4, NANOG, and OCT4 (A). Bisulfite sequencing analysis indicates the promoter region of OCT4 was demethylated in the hiPSCs and hESCs, but highly methylated in somatic cells (B). The qPCR demonstrates the expression of pluripotency genes comparable to hESCs (C). Karyotype analysis shows stable chromosomal integrity in all hiPSC lines.

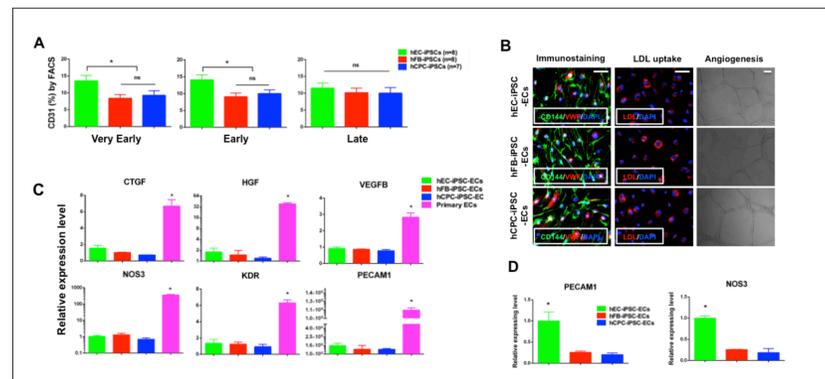


Figure 2 Characterization and comparison of EC differentiation in hiPSCs derived from different donor cell types. In very early ($p < 10$) and early ($p < 20$) passaged hiPSCs, EC-iPSCs showed higher EC differentiation propensity identified by the percentage of CD31 positive cells. However, in late passage ($p > 30$), all three hiPSCs displayed comparable EC differentiation efficiency (A). All iPSC-ECs expressed EC-specific markers CD144 and VWF. They also showed LDL uptake and formed tube-like structure in vitro (B). The EC markers were low-expressed in undifferentiated hiPSCs (C). In the process of EC differentiation (Day 5), PECAM1 (CD31) and NOS3 were expressed at higher levels in EC-iPSCs than other iPSCs, indicating that EC differentiation could be activated earlier in EC-iPSCs (D).

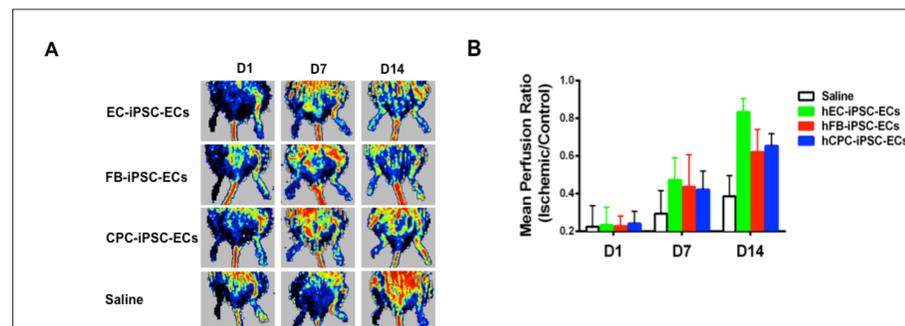


Figure 3 Laser doppler imaging of blood reperfusion in ischemic hindlimbs injected with iPSC-ECs. Transplanted EC-iPSC-ECs could generate greater revascularization and blood flow recovery than FB-iPSC-ECs and CPC-iPSC-ECs in two weeks after cell injection. Representative images (A) of bioluminescence assays and quantitative analysis (B) are shown.

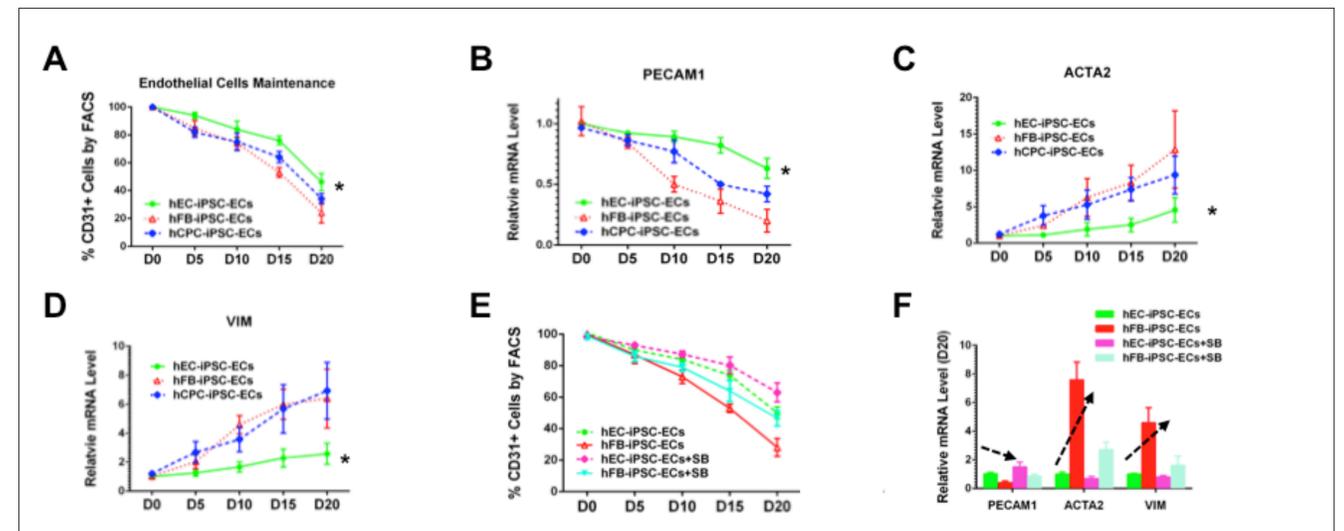


Figure 4 In vitro EC identity maintenance in iPSC-ECs. EC-iPSC-ECs sustained higher percentage of CD31+ cell population during long-time (20 days) culture than those of FB-iPSC-ECs and CPC-iPSC-ECs (A). Similarly, EC-iPSC-ECs maintained higher EC marker (CD31) gene expression but lower mesenchymal markers (ACTA2 and VIM) gene expression when compared with other iPSC-ECs during prolonged culture (B-D). TGF- β inhibitor (SB431542) boosted the CD31 expression in both EC-iPSC-ECs and FB-iPSC-ECs, though EC-iPSC-ECs still had higher CD31+ percentage than FB-iPSC-ECs in response to TGF- β inhibitor (E). Simultaneously, TGF- β inhibitor promoted EC marker (PECAM1) expression and blocked the mesenchymal markers (ACTA2 and VIM) expression in both EC-iPSC-ECs and FB-iPSC-ECs (F).

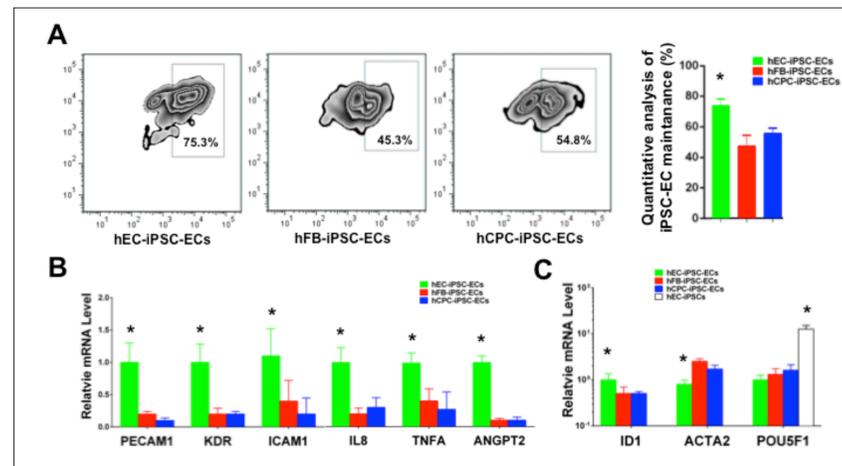


Figure 5 In vivo EC identity maintenance of iPSC-ECs in ischemic hindlimb mice. After cell transplantation in ischemic hindlimbs, the injected iPSC-ECs were retrieved by FACS in two weeks. The in vivo EC-iPSC-ECs show higher percentage of CD31/CD144 double positive population than FB-iPSC-ECs and CPC-iPSC-ECs (A). Single-cell qPCR analysis indicates that the recovered EC-iPSC-ECs expressed higher levels of EC markers than those of FB-iPSC-ECs and CPC-iPSC-ECs (B). In contrast, EC-iPSC-ECs had significantly less mRNA abundance of mesenchymal marker (ACTA2), but increased expression of ID1 that is required for the iPSC-ECs maintenance (C).

Conclusions

- Early-passage EC-iPSCs have higher differentiation propensity toward CD31+ endothelial cell lineage than FB-iPSCs and CPC-iPSCs. However, the biased differentiation propensity diminishes with extensive passaging.
- EC-iPSC-ECs show higher EC-specific marker gene expression and EC identity maintenance with extensive culture than FB-iPSC-ECs and CPC-iPSC-ECs.
- Upon transplantation, EC-iPSC-ECs exhibit greater revascularization capacity than those of FB-iPSC-ECs and CPC-iPSC-ECs in a mouse ischemic hindlimb model.
- In vivo transplanted EC-iPSC-ECs maintain higher percentage of CD31+ population and stronger EC marker gene expression.

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