Generation of Multipotent Lymphoid-Competent CD34+ Hematopoietic Progenitor Cells from Human Induced Pluripotent Stem Cells

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OVERVIEW

- Hematopoietic progenitor cells (HPCs) can be generated from induced pluripotent stem cells (iPSC) in a scalable protocol with high yield and purity.
- iPSC derived HPCs resemble those from the early (5-6 week) human embryo in the aorta-gonad-mesonephros (AGM) region.
- iPSC derived HPCs are multipotent and lymphoid-competent, generating erythroid-myeloid cell types in colony-forming assays, and multiple lymphoid cell types in downstream differentiation.

METHODS

iPSC Differentiation

iPSCs were differentiated to CD34+ HPCs in scalable agitated suspension cultures and MACS-enriched CD34+ cells were cryopreserved. The potential of fresh or cryopreserved CD34+ HPCs was assessed by colony-forming assays (CFU) in MethoCult and by directed differentiation to lymphoid lineages. Cell phenotypes were monitored throughout differentiation by flow cytometry and at select timepoints by single cell RNA sequencing (scRNAseq).

scRNAseq processing

Trimming, alignment, demultiplexing and gene counts were generated from FASTQ files using CellRanger and gene counts matrices normalized using Seurat[1] (4.0.1). Doublets were identified and removed, and dead cells removed by filtering those with greater than 5% mitochondrial reads.

Cell annotation and ontogeny assignment

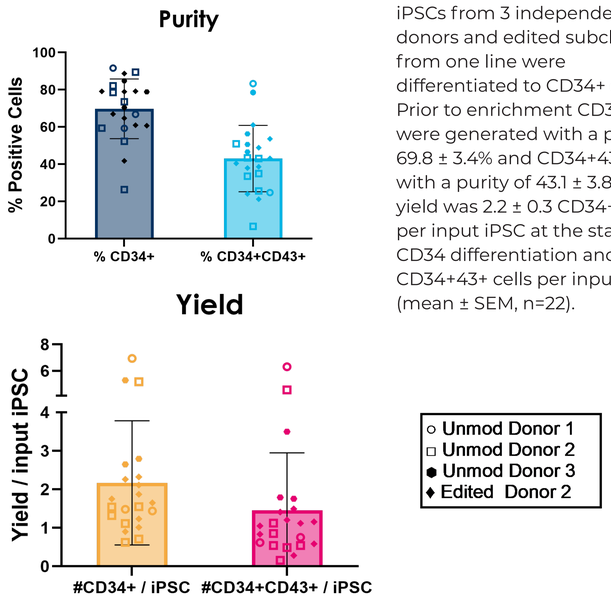
Cell type labels were assigned using ACTINN[2] with the training dataset and input parameters as reported in Calvanese et al.[3]

Gene set enrichment

Single sample GSEA scores were calculated at the single cell level using GSVA (1.40.1) using the 20-gene signatures representing T cell maturation in developing thymocytes reported in Park et al.[4]

RESULTS

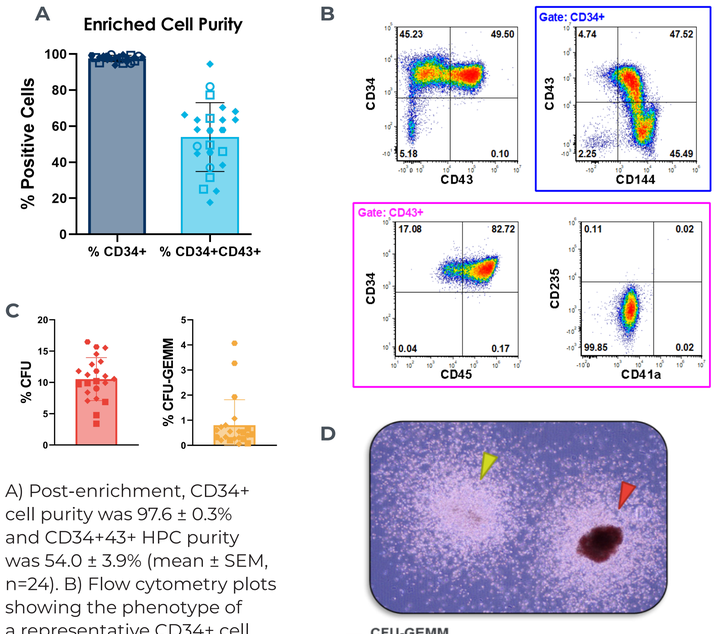
Figure 1. CD34+ hematopoietic progenitor cells are robustly generated from induced pluripotent stem cells



iPSCs from 3 independent donors and edited subclones from one line were differentiated to CD34+ cells. Prior to enrichment CD34+ cells were generated with a purity of 69.8 ± 3.4% and CD34+43+ HPCs with a purity of 43.1 ± 3.8%. The yield was 2.2 ± 0.3 CD34+ cells per input iPSC at the start of CD34 differentiation and 1.5 ± 0.3 CD34+43+ cells per input iPSC (mean ± SEM, n=22).

RESULTS - CONT'D

Figure 2. iPSC derived CD34+ banks contain high frequency of HPCs



A) Post-enrichment, CD34+ cell purity was 97.6 ± 0.3% and CD34+43+ HPC purity was 54.0 ± 3.9% (mean ± SEM, n=24). B) Flow cytometry plots showing the phenotype of a representative CD34+ cell bank. Approximately half of the CD34+ cells have differentiated to CD43+ HPCs with decreasing levels of CD144 (VE-cadherin). Of the CD43+ HPCs, most co-express CD45, and do not express CD235 or CD41a. C) In the CFU assay, 10.5 ± 0.7% of enriched CD34+ cells generated colonies, and 0.8 ± 0.2% of cells formed multipotent GEMM colonies. D) Representative image showing CFU-GM and CFU-GEMM colonies.

Figure 3. iPSC derived CD34+ cells resemble hematopoietic progenitors and endothelial cells from tissues in the early human embryo

iPSC derived CD34+ HPCs (n=3) were assessed by scRNAseq and compared to those from in vivo hematopoietic tissues in the human embryo described in Calvanese et al.[3] iPSC derived CD34+ cells most resembled endothelial and hematopoietic cell types found in Carnegie Stage (CS) 10-15 human embryos.

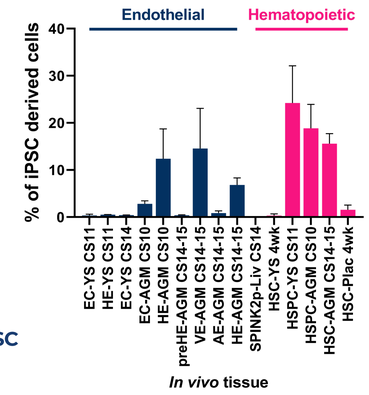


Figure 4. Lymphoid potential of iPSC derived CD34+ cells

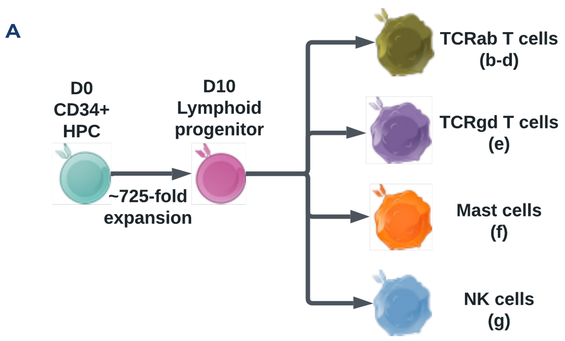
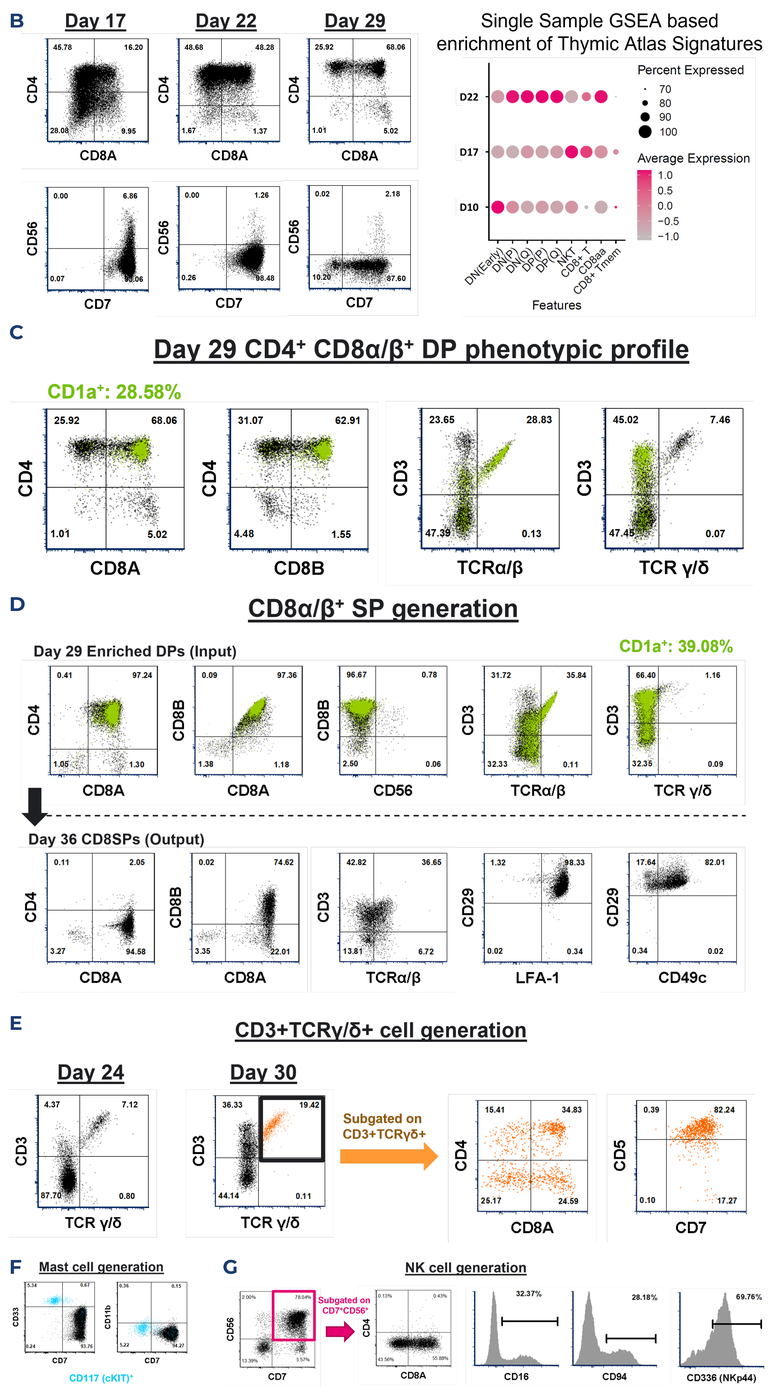


Figure 4. Lymphoid potential of iPSC derived CD34+ cells - CONT'D



A) Overview of lymphoid cell types generated from iPSC derived CD34+ HPCs. B-D) Timecourse of differentiation to TCRαβ T cells. B) Gain of T lineage fate between D10-29 of differentiation producing primarily CD4+CD8+ double positive (DP) cells. C) T cell commitment was further shown by expression of CD1a, which was enriched in CD4+CD8ab+ DP cells and CD3+ cells expressing TCRαβ. D) Enriched DP cells were successfully transitioned to CD8+ single positive (SP) cells. E) Generation of TCRγδ T cells, which were observed in CD4-CD8- DN, CD4+CD8+ DP and both CD4+ ISP and CD8+SP cell populations. F) Generation of CD7-CD33+CD117+CD11b- mast cells. G) Generation of CD7+CD56+ natural killer (NK) cells co-expressing NK markers CD16, CD94 and NKp44.

CONCLUSIONS

- iPSC derived CD34+ HPCs are multipotent, producing lymphoid cell types including TCRαβ and TCRγδ T cells, mast cells, and NK cells.
- CD8SP T cells expressing CD3 and TCRαβ are generated via a DP stage in a process that mimics a natural thymic developmental progression.

1. Hao, Y et al. Integrated analysis of multimodal single-cell data. Cell June 24 (2021).
2. Ma, F. & Pellegrini, M. ACTINN: automated identification of cell types in single cell RNA sequencing. Bioinformatics 36, 533–538 (2019).
3. Calvanese, V. et al. Mapping human haematopoietic stem cells from haemogenic endothelium to birth. Nature 1–7 (2022) doi:10.1038/s41586-022-04571-x.
4. Park, J.-E. et al. A cell atlas of human thymic development defines T cell repertoire formation. Science 367, eaay3224 (2020).