CD82 IS SUFFICIENT TO UNIQUELY IDENTIFY PLURIPOTENT STEM CELL-DERIVED HEMOGENIC ENDOTHELIUM WITH THE HEMATOPOIETIC LINEAGE POTENCY TO GIVE RISE TO BONA FIDE LYMPHOCYTES

A. YZAGUIRRE, C. DEGE, D. SIVALINGAM, R. SCHNELLMANN, A. BAGRI, A. WITTY, B. VALAMEHR

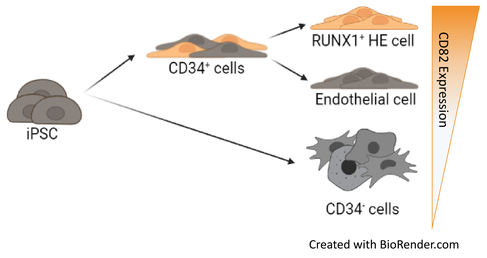
Fate Therapeutics, Inc., San Diego, CA

BACKGROUND

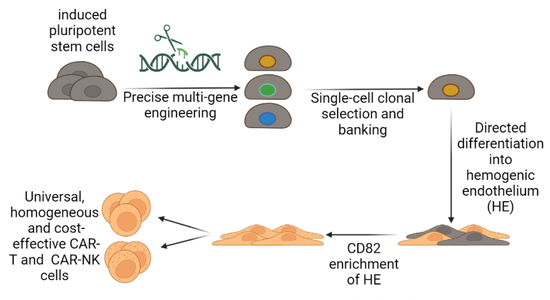
Chimeric antigen receptor (CAR)-T and natural killer (NK) cells represent compelling opportunities for the treatment of hematological malignancies and solid tumors. However, autologous and donor-derived allogeneic CAR-T and NK cell therapies are limited based on the quantity and quality of the starting engineered cells. We previously described our off-the-shelf induced pluripotent stem cell (iPSC)-derived CAR-T (CAR-iT) and CAR-NK (CAR-iNK) cell platforms that eliminate some of the hurdles associated with patient-derived cells by creating a homogenous cell product that is derived from a renewable bank of iPSC master cell lines. A major challenge in the manufacturing of iPSC derived immune cells is the derivation of hemogenic endothelium (HE), which is the rare and transient direct precursor of hematopoietic cells, making its *in vitro* specification from iPSCs inefficient and challenging. Furthermore, there is not a reliable marker or set of markers for the identification of HE in culture. HE identification has traditionally relied on detection of the transcription factor RUNX1 and the establishment of reporter cell lines, neither of which are suitable for the manufacture of HE cells destined to become therapeutic immune cells.

We report here the identification of tetraspanin, CD82 as a novel HE marker that is sufficient for the isolation of an enriched population of HE with the potential to give rise to bona fide iNK and iT cells. The identification of CD82 for the enrichment of iPSC derived HE is a critical step to the development of definitive hematopoiesis with ability to give rise to all lineages of the hematopoietic compartment and facilitates the scaled and consistent manufacture of high-quality T and NK cells for off-the-shelf therapeutic applications.

CD82 EXPRESSION DURING HEMATOPOIESIS



OFF-THE-SHELF PLATFORM



RESULTS

CD82 IDENTIFIED AS CANDIDATE HE MARKER VIA ANTIBODY ARRAY AND scRNA-SEQ

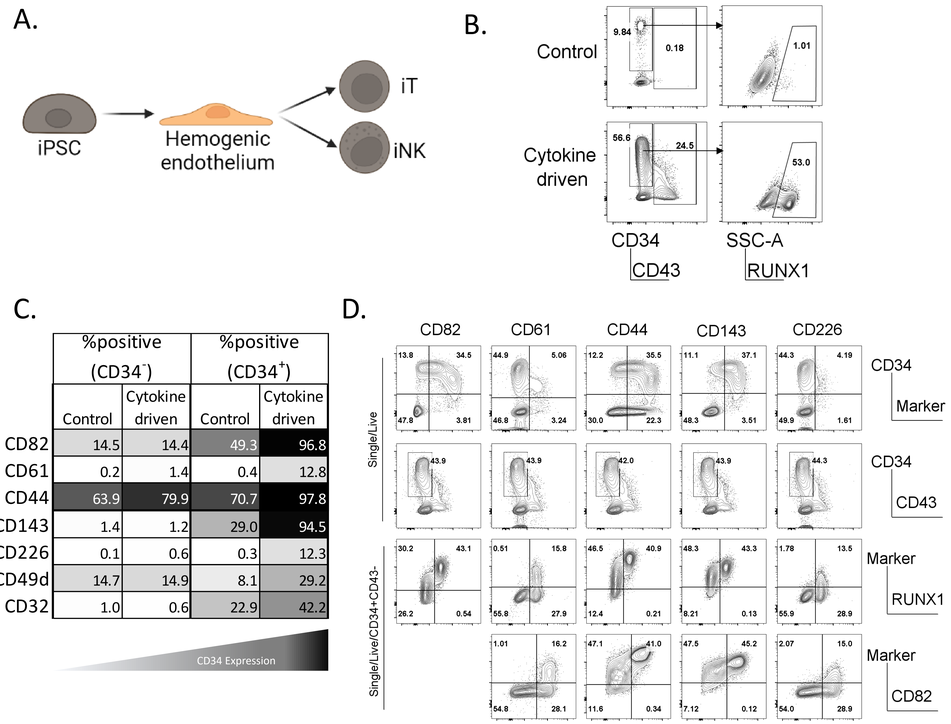


Figure 1. A) Schematic diagram of the differentiation of induced pluripotent stem cells (iPSC) into hemogenic endothelium (HE) and subsequently induced T (iT) and natural killer (iNK) cells; D, day of differentiation. Schematic created with BioRender.com. B) Flow cytometric analysis of cells, differentiated under control conditions and with cytokines that promote the specification of RUNX1+ HE (Cytokine driven), cells are gated on single/live events. C) Summary of candidate HE surface markers identified via a BioLegend LEGENDScreen. Selected candidates have a higher percentage of expression within the CD34+ subset of cytokine driven cells compared to control cells. D) Flow cytometric analysis of cytokine driven cells comparing expression of HE candidate markers with RUNX1 and CD82. E) scRNAseq analysis of cytokine driven cells that were purified using CD34 magnetic activated cell sorting. UMAP visualization of the expression of curated feature genes used for the identification of cell clusters. Each dot represents one cell. F) Transcriptomic identification of cell populations visualized by UMAP. Violin plot of CD82 expression within each scRNA-seq cell cluster. EC, endothelial cell.

CD82 IDENTIFES HEMOGENIC ENDOTHELIUM THAT GIVES RISE TO BONA FIDE NK AND T CELLS

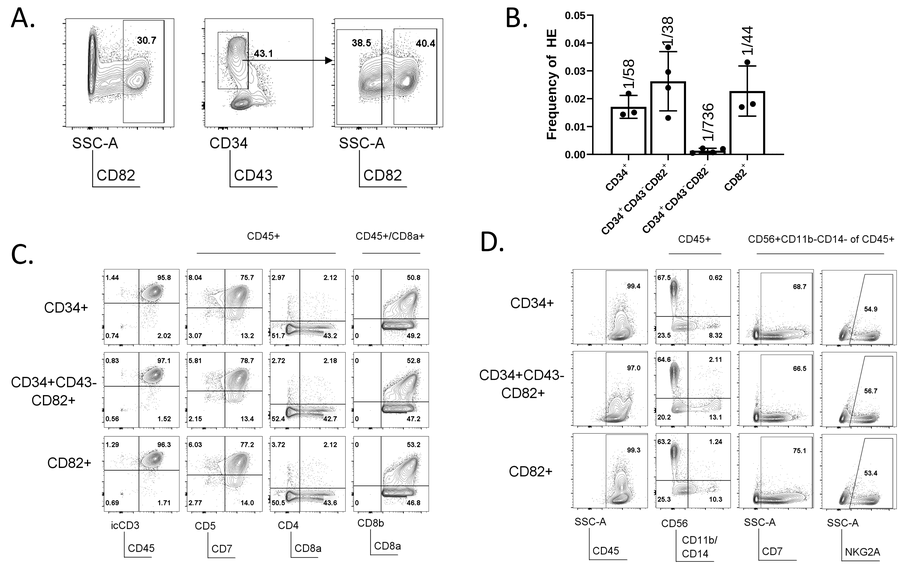


Figure 2. A) Flow cytometric analysis of cytokine driven cells demonstrating populations sorted for limiting dilution hemogenic endothelium assays. B) Frequency of HE within cytokine driven populations that were fluorescence-activated cell sorted (FACs) based on indicated markers on x-axis (mean±SD). Average HE frequency is indicated above columns. Frequencies calculated using ELDA software. C) Flow cytometric analysis of iT cells derived from cytokine driven populations FAC sorted based on the markers indicated above flow plots. icCD3, intracellular CD3. D) Flow cytometric analysis of iNK cells derived from cytokine driven populations FAC sorted based on the markers indicated above flow plots.

SELECTION OF CD82 CELLS IS SUPERIOR TO CD34 FOR THE ENRICHMENT OF RUNX1+ HE CELLS

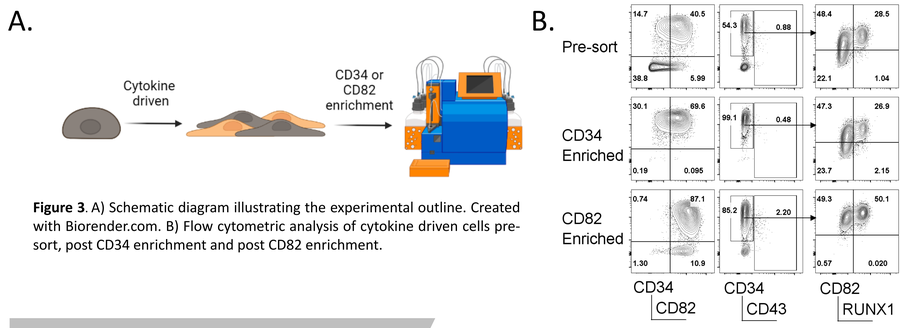


Figure 3. A) Schematic diagram illustrating the experimental outline. Created with Biorender.com. B) Flow cytometric analysis of cytokine driven cells pre-sort, post CD34 enrichment and post CD82 enrichment.

CONCLUSION

- We identified several surface markers that were expressed by a higher percentage of CD34+ endothelial cells compared to control CD34+ cells.
- We identified CD82 as a novel candidate HE marker, with expression uniquely restricted to the CD34+ endothelial population and positively correlating with RUNX1 expression, an important marker of hematopoiesis.
- We show that CD82 is sufficient to enrich for HE derived from iPSCs with the potential to give rise to definitive lymphoid cells, improving the consistency, potency and manufacturability of allogeneic, off-the-shelf, multiplexed-engineered iNK and iT cells.