The STEMdiff™ Hematopoietic Kit Reproducibly Generates Functional Hematopoietic Progenitor Cells from Human Pluripotent Stem Cells

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Introduction

Hematopoietic cells generated from human pluripotent stem cells (hPSCs) can be used to model blood diseases and may become an alternate source of blood cells for transplantation. However, robust methods to differentiate hPSCs to hematopoietic progenitor cells (HPCs) have been difficult to develop. The STEMdiff™ Hematopoietic Kit reproducibly generates HPCs expressing key hematopoietic cell surface markers and transcription factors under serum- and feeder-free conditions. The functionality of the generated HPCs can be assessed in semi-soild cultures designed specifically to support balanced erythroid and myeloid colony growth from hPSC-derived HPCs.

Methods

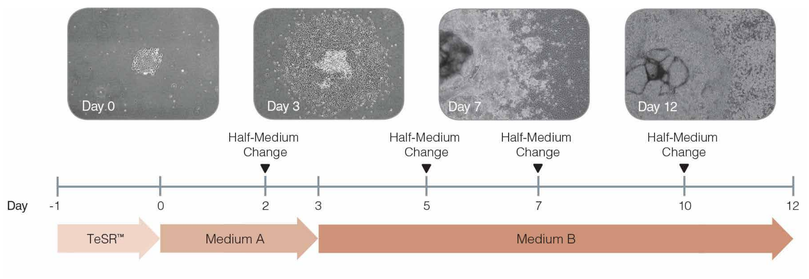

Figure 1. Protocol for use of the STEMdiff™ Hematopoietic Kit. Briefly, 10 - 20 hPSC aggregates per cm^2 were plated on Corning® Matrigel® in TeSR™ medium. The cells were then sequentially incubated in two STEMdiff™ differentiation media and harvested on day 10 or 12. HPCs have been reproducibly generated from multiple embryonic (H1, H9) and induced pluripotent (WLS-1C, STiPS-F016, STiPS-M001, STiPS-B004) stem cell lines.

Results

Human PSC-Derived HPCs Express Key Hematopoietic Cell Surface Markers and Transcription Factors

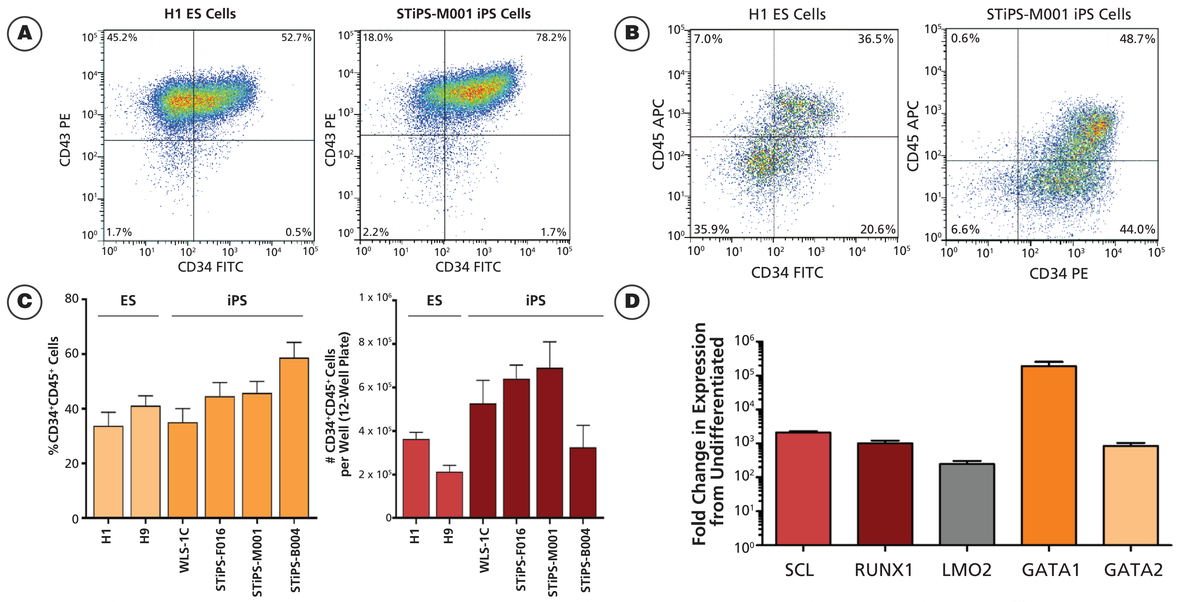

Figure 2. Characterization of Hematopoietic Progenitor Cells Derived from hPSCs Using STEMdiff™ Hematopoietic Kit. At day 12 of differentiation, most HPCs were detected in the supernatant fraction of the culture. The cells were harvested from the supernatant and assessed by flow cytometry **(A-C)** and by qPCR **(D)** for expression of key hematopoietic markers. **A)** The embryonic pan-hematopoietic marker CD43 was expressed on 96.4% ± 0.5 of day 12 supernatant cells (mean ± SEM, n = 8). Representative flow cytometry plots are shown. **B)** HPCs were detected by co-expression of CD45 and CD34 as shown on representative flow cytometry plots. **C)** Bar graphs summarize the percentage and yield of CD34$^+$CD45$^+$ HPCs per well of a 12-well plate across 6 hPSC lines (mean ± SEM, n = 3 - 17 per cell line). **D)** Transcription factors involved in developmental hematopoiesis were highly upregulated during differentiation. Expression by qPCR was normalized to housekeeping genes 18S and TBP, then day 12 samples were normalized to undifferentiated controls (mean ± SEM, n = 8).

Erythroid Colonies Derived from hPSC Cell Lines Express Primarily Embryonic and Fetal Globins

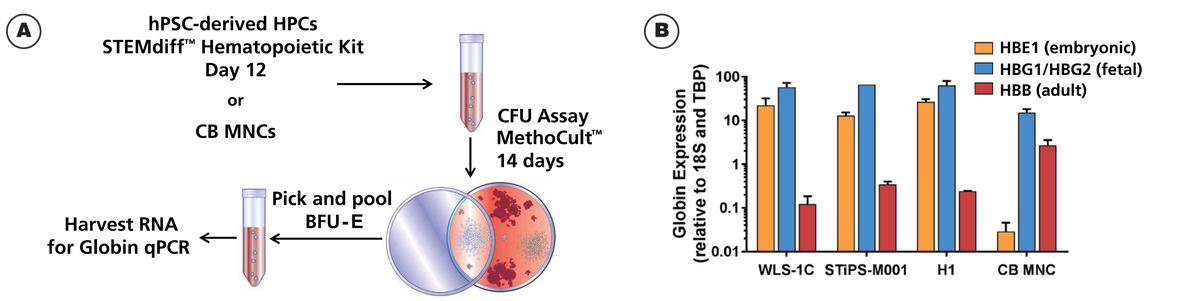

Figure 3 . hPSC-Derived Erythroid Colonies Express Primarily Embryonic and Fetal Globins. A) Schematic showing how samples were prepared for globin expression analyses by qPCR. **B)** Globin expression was normalized to expression of housekeeping genes 18S and TBP. hPSC-derived erythroid colonies from 1 ES (H1) and 2 iPS (WLS-1C, STiPS-M001) cell lines expressed primarily embryonic (HBE1) and fetal (HBG1/HBG2) globins. In contrast, erythroid colonies generated from cord blood mononuclear cells (CB MNC) expressed primarily fetal and adult (HBB) globins (mean ± SD, n = 2).

HPCs Derived from hPSCs are Capable of Multilineage Differentiation in Semi-Solid Medium

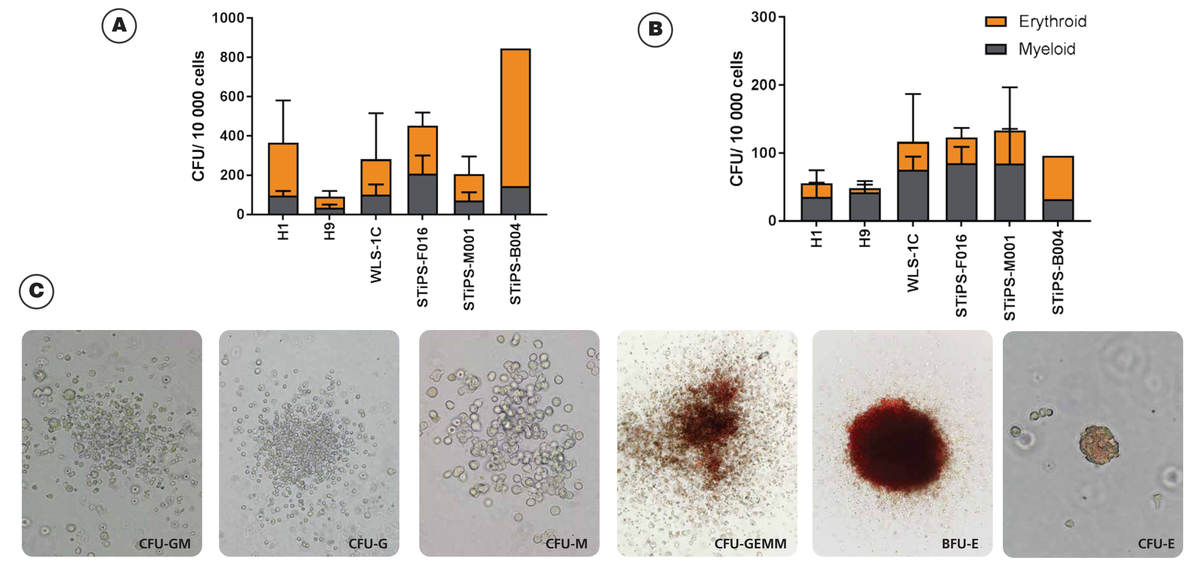

Figure 4. The Frequency of Hematopoietic Colony-Forming Unit (CFU) and Colony Type Distribution. The colony-forming potential of PSC-derived HPCs was assessed in serum-free MethoCult™ medium (H4636) designed specifically to support balanced erythroid and myeloid colony growth from hPSC-derived HPCs. **A - B)** Bar graphs summarize the frequency of CFU per 10,000 cells and show distribution of myeloid (grey) and erythroid (combined CFU-E and BFU-E, orange) colonies derived from differentiated cells harvested on day 10 **(A)** and 12 **(B)** (mean ± SEM, n = 1 - 5 per cell line). **C)** Representative images of colonies (40X magnification) are shown.

Human PSC-Derived HPCs can be Effectively Expanded and Differentiated to Red Blood Cells

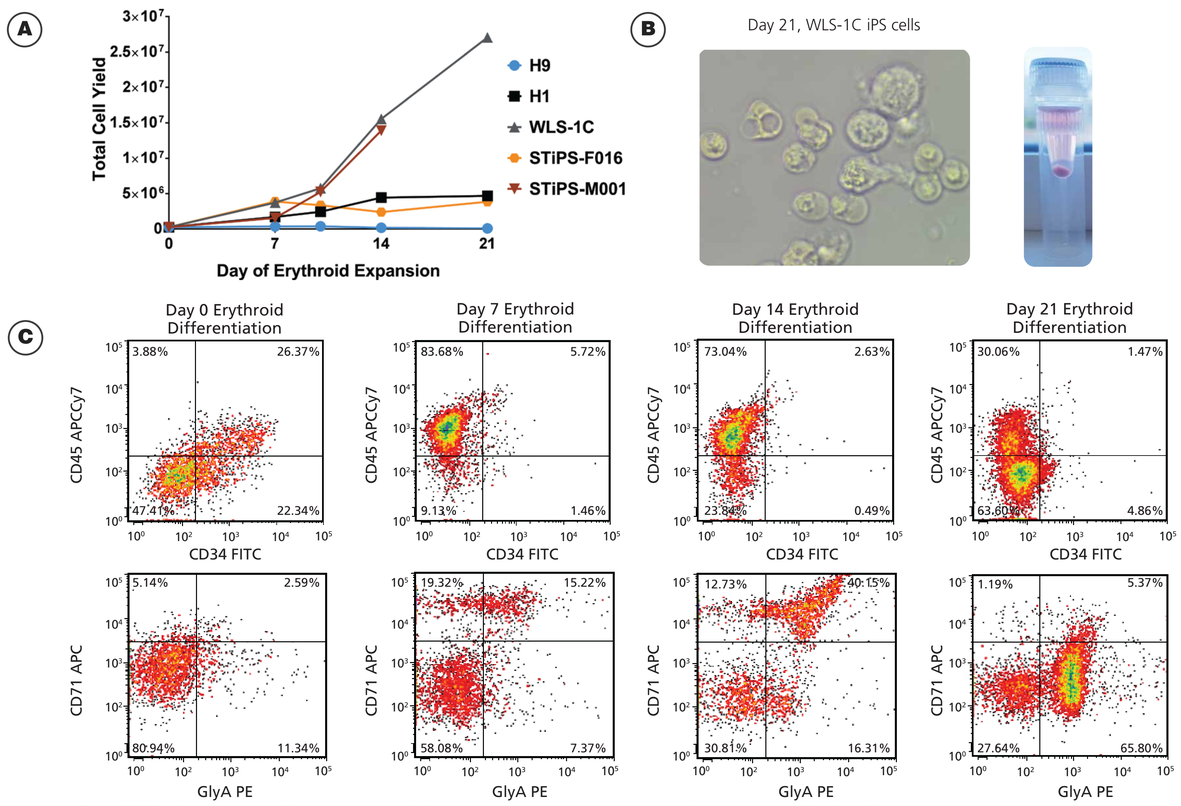

Figure 5. hPSC-Derived HPCs Further Differentiate Into Erythroid Progenitor Cells. Cells were harvested on day 12 of hematopoietic differentiation, seeded at 2.5 x 10^5 cells/mL into StemSpan™ SFEM II with Erythroid Expansion Supplement, and cultured for up to 21 days. **A)** Expansion over 21 days was between 4- and 56-fold. **B)** Morphology (left) and the red color of the cell pellet (right) cultured for 21 days indicate effective erythroid differentiation and hemoglobinization. **C)** Progressive erythroid differentiation during the culture can be observed by changes in expression of CD45, CD34, CD71 and Glycophorin A markers. After 21 days of culture in erythroid expansion conditions, 20 - 70% of cells were CD71$^-$GlyA$^+$CD45$^-$ and displayed low FSC and SSC (not shown) as assessed by flow cytometry. Representative FACS plots from WLS-1C cells are shown.

Summary

- HPCs can be robustly and reproducibly generated from multiple human ES and iPS cell lines in 10 -12 days under serum-free and feeder-free culture conditions using the STEMdiff™ Hematopoietic Kit
- hPSC-derived HPCs express hematopoietic cell surface markers and transcription factors, are functional and capable of myeloid and erythroid differentiation
- The colony-forming unit (CFU) potential of hPSC-derived HPCs can be assessed in MethoCult™ H4636, a semi-solid, serum-free medium designed specifically to support optimal and balanced erythroid and myeloid colony growth from hPSC-derived hematopoietic cells