Enforced expression of TCF3 or BCL11B enhances T progenitor differentiation from human induced pluripotent stem cells

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Background and Significance

Induced pluripotent stem cells (**iPSC**) derived hematopoietic stem and progenitor cells (**HSPCs**) are being developed clinically as an attractive alternative source of "off the shelf" T lymphocytes for cellular therapy for human malignancies and GVHD. **However, iPSC-derived HSPCs are significantly less efficient at generating functional T cells compared to other phenotypically similar sources such as umbilical cord blood-derived (UCB) HSPCs *in vitro*.**[1] A rigorous understanding of what underpins this difference is lacking in the literature and is a significant barrier to clinical translation. To address this deficiency, we have established a monolayer differentiation system for human iPSCs that reproducibly and efficiently generates HSPCs with broad myelolymphoid potential via a hemogenic endothelial intermediate.

Hypothesis: iPSC-derived HSPCs have inferior T cell potential compared to UCB-derived equivalents due to altered regulation of key lymphoid transcription factors TCF3 and BCL11B, leading to competing populations of lineage-biased progenitors

Results

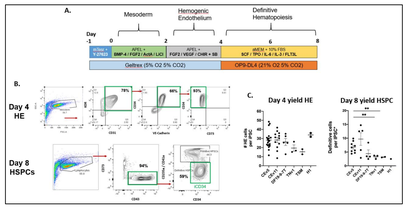

Figure 1 – Monolayer protocol efficiently generates definitive hematopoietic progenitors with myelolymphoid potential from human induced pluripotent stem cell lines. iPSCs from a variety of sources were tested using a monolayer HSPC differentiation protocol. **A)** Outline of differentiation protocol used for this studies. **B)** Representative flow cytometry plots demonstrating cell surface phenotype of hemogenic endothelial (HE) cells generated at Day 4 and phenotype of hematopoietic stem and progenitor cells (HSPCs) generated at Day 8. **C)** Yield of D4 HE and Day 8 HSPCs expressed as a HE or definitive HSPC per iPSC input at D-1 for lines tested. CEv5, CEv11 and DF19-9 were derived from Fibroblasts. TnV1, Tsm were derived from T cells. H1 is a human embryonic stem cell line. (**= p<0.05)

Results

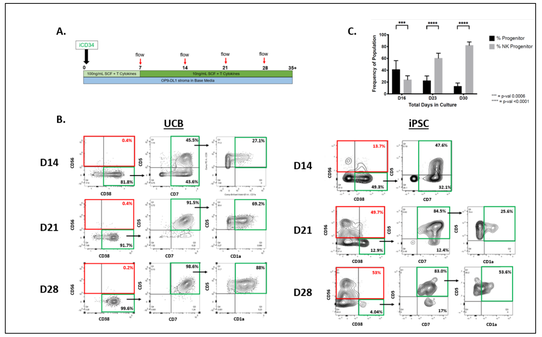

Figure 2 – iPSC-derived HSPCs placed into T progenitor promoting conditions develop canonical markers of T progenitor differentiation, however competing population of CD56+ NK/ILC progenitors compromises development. A) Outline of OP9-DL1 stromal cell co-culture method of T lymphoid differentiation from HPSCs. **B)** Representative flow plot demonstrating acquisition of canonical markers of T lymphoid specification in both UCB and iPSC-derived HSPCs (green boxes) and the outgrowth of a strongly CD56+ alternative cell type found only in iPSC-derived cultures. **C)** Quantitation of CD56+ progenitor outgrowth in iPSC-derived HSPC cultures showing increasing CD56+ frequency at expense of T progenitor frequency.

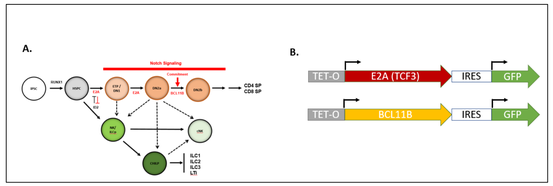

Figure 3 – Regulation of critical transcription factors governs T vs NK/ILC lineage fate decisions. We hypothesized that altered regulation of critical transcription factors that enforce a T phenotype and inhibit a NK/ILC phenotype might be responsible for the CD56+ cells generated from iPSC-HPSC cultures. **A)** Outline of developmental relationship between T and NK/ILC cell lineages. The critical transcription factors TCF3 (E2A) and BCL11B and their role in T cell commitment are highlighted. **B)** Overview of Piggybac doxycycline inducible transcription factor expression vectors that were constructed.

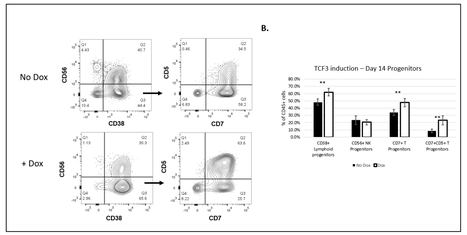

Figure 4 - Inducible overexpression of TCF3 during T cell commitment enhances T progenitor commitment from human iPSC HSPCs at Day 14 of differentiation. TCF3 expression was induced using doxycycline during T progenitor differentiation. **A)** Representative flow cytometry plot demonstrating enhanced CD38+CD56- commitment and CD7+ as well as CD5+CD7+ Tprogenitors at Day 14 of T cell differentiation with dox induced TCF3 expression. **B)** Quantitation of n=4 experiments demonstrating significant increases in the frequency of CD38+ lymphoid progenitors as well as increases in total CD7+ and CD5+CD7+ Tprogenitor populations. There was no decrease in CD56+ NK progenitors. (** = p<0.05)

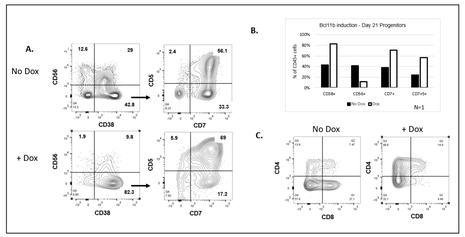

Figure 5 - Inducible overexpression of BCL11B during T cell commitment enhances T progenitor commitment and decreases NK cell commitment from human iPSC HSPCs at Day 21 of differentiation. BCL11B expression was induced using doxycycline during T progenitor differentiation. **A)** Surface phenotype of inducible BCL11B engineered iPSC line with and without addition of doxycycline during T cell differentiation, measured at day 21 of differentiation demonstrating enhanced frequency of CD38+ acquisition as well as total CD7+ and CD7+5+ T progenitor commitment. **B)** Quantitation of critical population subsets showing enhancement of CD38+ lymphoid progenitors, a decrease in CD56+ NK/ILC population and increase in CD7+ and CD7+CD5+ T progenitors. **C)** Gated on CD5+CD7+ cells, demonstrating the relative frequency of CD4 and CD8 expression amongst the CD45+CD38+CD56-CD5+CD7+ T progenitors at D21. This shows enhanced commitment to canonical CD4+ intermediate single positive and double positive in the +dox group and reduction in noncanonical CD8+ intermediate single positive cells.

Conclusion

- Monolayer differentiation protocol for human iPSCs produces efficient and reproducible generation of HSPCs with lymphoid potential
- iPSC-derived HSPCs follow canonical phenotypic stages of T lymphoid differentiation similar to UCB-derived HSPCs but also generate a competing population of CD56+ NK/ILC progenitors that impede T cell differentiation.
- Inducible expression of TCF3 and BCL11B during T cell differentiation enhances T cell commitment at D14 and D21 respectively. BCL11B expression also reduced commitment to the CD56+ alternative lineage and enhanced canonical CD4 commitment.
- These data support the hypothesis that insufficient activation of Pro-T lymphoid commitment genes during T cell development from iPSC may explain the reduced efficiency of T cell generation

Future Directions

- Explore the transcriptional state of iPSC derived HSPCs compared to UCB-HSPCs with single cell RNA-seq
- Identify critical surface receptors, transcription factors and signaling pathways upstream of TCF3 and BCL11B that regulate their expression during iPSC T cell commitment
- Test enhanced iPSC-derived HSPC protocols for enhanced T cell efficiency and yield

Funding

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References: 1. Brauer et al. Trends in Immunology 2016