Lineage Tracing of HoxB5 Cells of the Yolk Sac and their Contribution to the Adult Hematopoietic System

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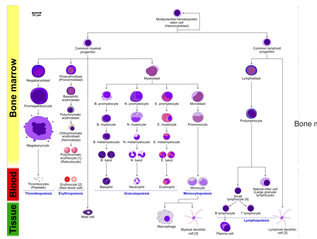
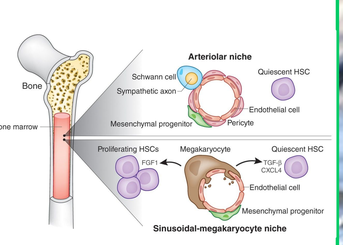
Abstract

Understanding the origins of hematopoietic stem cells has been a challenge due to the lack of a marker specific to this cell type. Recently, our lab has functionally proven that HoxB5 is a unique marker for long-term HSCs in adult murine models. Whether HoxB5 is also a marker for HSCs in development, has not been studied. Here, we are labeling HoxB5+ cells found in the yolk sac blood islands at E7.5 by using a genetic tool we generated. We will then analyze all marked cells at several time points during development and adulthood. This project will discover if HoxB5+ cells from the yolk sac contribute to the adult hematopoietic system and the LT-HSC pool.

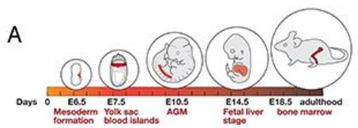
Introduction

- Hematopoietic Stem Cells give rise to and maintain the entire blood system. In adulthood, they are found primarily in the bone marrow located near sinusoidal membranes and in the spleen.

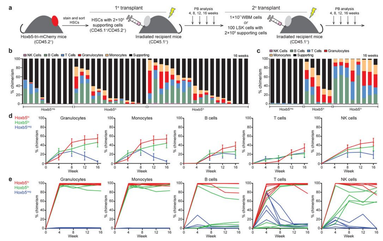




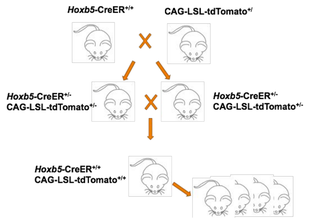
- Hematopoiesis occurs in three waves: the primitive wave at E7.5 when the yolk sac blood islands are formed, pro-definitive wave at E8.5 when erythromyeloid progenitors are formed and the definitive wave at E10.5 when HSCs emerge from hemogenic endothelial cells of the aorta gonad mesonephros.[2]



- HoxB5 is a gene that has been functionally proven by members of our lab to identify and enrich for long-term hematopoietic stem cells.[3]



Methods



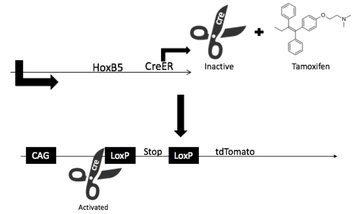
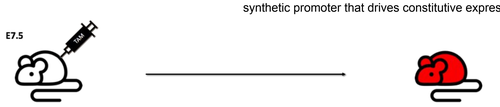
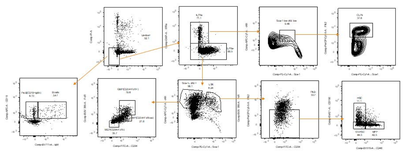


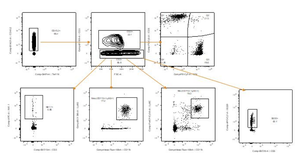
Figure 1: HoxB5 expression drives the expression of CreER. Cre is inactive unless in the presence of tamoxifen. It then makes nicks at LoxP sites. CAG is a synthetic promoter that drives constitutive expression.



Gating Strategy: Bone Marrow



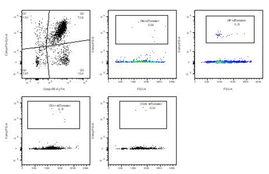
Gating Strategy: Peripheral Blood

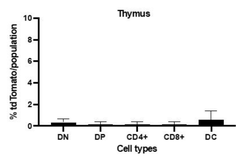


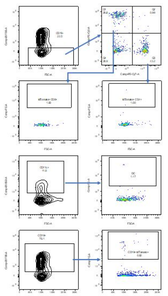
Results

Figure 2: FACS analysis of the Thymus tissue. There are small populations of CD4, CD8, Double Positive, and Double Negative T Cells that are positive for tdTomato.

Thymus







Spleen

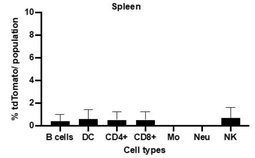
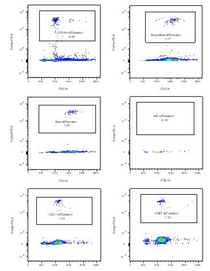


Figure 3: FACS analysis of the spleen. Here, you can see that small populations of Dendritic Cells, CD4 and CD8 T Cells, and Natural Killer T Cells are positive for tdTomato. Though, we did not see Monocyte or Neutrophil populations positive for tdTomato.

Results

PB



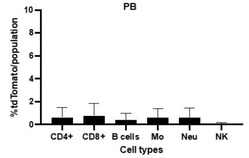
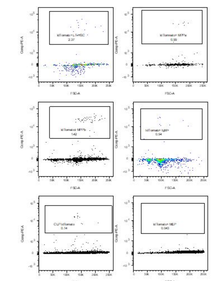


Figure 4: FACS analysis of the peripheral blood. Here we observed a small percentage of CD4 and CD8 T Cells, B Cells, Monocytes and Neutrophils positive for tdTomato. We also observe an extremely small number of Natural Killer T Cells positive for tdTomato.

BM



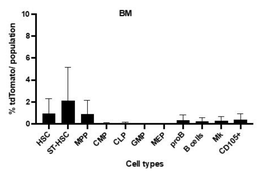


Figure 5: FACS analysis of the bone marrow. Here we can see that there is a population of LT and ST-HSCs that are positive for tdTomato. We also observed pro-B cells, B cells, Megakaryocytes and a very small population of CLP and CMP cell types marked by tdTomato. There is also a population of CD105+ cells marked by tdTomato, a marker common to endothelial cells.

Conclusions

- HoxB5 cells found in the yolk sac blood islands do contribute to the hematopoietic system and the LT-HSC pool
- Small number of marked cells could be from inadequate activation of Cre-recombinase from low dose of tamoxifen. Another explanation is that yolk sac HoxB5 cells have low contribution to the LT-HSC pool.
- Small number of CD105+ cells were identified in FACS analysis. We are not sure why this is, but it could be that yolk sac HoxB5 cells differentiate into the hemogenic endothelium, which differentiates into both endothelial cells and hematopoietic stem cells.
- We are currently optimizing a mode of administration where we inject 4-hydroxytamoxifen directly into the yolk sac.

Acknowledgments

Big thank you to the Weissman Lab, they were a great group of people to work with.

Thank you Dr. Jenny Cappuccio, Dr. Amy Sprowles and Dr. Brigitte Blackman for giving me this opportunity to follow my dream of becoming a scientist.

Our grant: CIRM Bridges 3.0 #EDUC2-12620

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Margaret H. Baron, Joan Isern, Stuart T. Fraser; The embryonic origins of erythropoiesis in mammals. *Blood* 2012; 119 (21): 4828–4837. doi: https://doi.org/10.1182/blood-2012-01-153486 - image of HSC development