Establishment of a Hematopoietic Stem Cell Generating System in Mouse and Human Fibroblasts

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Abstract— **Hematopoiesis is a physiologic process wherein hematopoietic stem cells give rise to lineage-restricted progenitors that subsequently give rise to all blood cells. This project seeks to identify the transcription factors and environmental conditions necessary for inducing a hematopoietic program in mouse and human fibroblasts. Thus far, it has been determined that Gata2, Gfi1b, cFos and Etv6 are sufficient for induction of a hemogenic process.**

I. Methods

To identify the optimal set of transcription factors (TFs) that can induce a hematopoietic fate in somatic mouse fibroblasts, factors known to be highly expressed in HSCs were first identified. A set of these TFs were cloned into pMX retroviral vectors and selected by using a human CD34 reporter mouse (34/H2BGFP), obtained from transgenic (genetically-modified) mice. TFs capable of activating a hemogenic (blood cell-producing) program would activate the human CD34 reporter, causing cells to express Green Fluorescent Protein (GFP). In doing so, a set of 18 TFs with HSC-producing potential was identified (Pereira et al., 2013).

To establish the set of TFs critical to generating HSCs *de novo*, the systematic removal of certain TFs was commenced. The first 4 TFs to be removed were known to be expressed in dormant and active HSCs. The 14 TF mouse embryonic fibroblasts (MEFs) were co-cultured with AFT024 (cell line), with and without cytokines (proteins which impact cell interactions). GFP+ and GFP- colonies were quantified for 18 days. An increase in the total GFP+ colony numbers was noted, as with GFP- colonies that appeared without cytokines.

In order to refine the cocktail so as to include only TFs critical to HSC induction, the impact of the removal of each TF on the number of 34/H2BGFP+/- colonies produced was assessed. Upon doing so, the TF combination responsible for hematopoiesis was identified. The removal of Etv3, HoxA9, and Rrd1each increased GFP+ colony frequency. The removal of Lyl1, Scl, Mllt3, and Meis1 each had no impact on the GFP+ colony frequency. The removal of Gata2, Gfi1b or cFos, *dramatically decreased* GFP+ colony production. Etv6 was revealed to be unnecessary for HSC induction, but optimizes the process. *It was determined that Gata2, Gfi1b, cFos and Etv6 are sufficient for 34/H2BGFP activation, signifying a hemogenic program* (Pereira et al., 2013).

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II. Results and Conclusion

Unsupervised hierarchal clustering (statistical analysis) placed day 40 GFP+ cells generated without cytokines closest to *bona fide* adult HSCs. Furthermore, a decrease in the expression of fibroblast-specific genes, such as Vim, Acta2, Fn1, and Fbn2 over the course of days 0 and 20 was observed. Finally, high levels of Prom1 and Ly6a as well as an increase in expression of KitL, Csfr, CD43, and I13r were also observed. So long as Gata2, Gfi1b, and cFos were present within the GFP+ TF groups, global expression profiles (gene-expression analysis results) were similar, therefore indicating that the additional factors serve to impact efficiency, but are otherwise not integral to producing a hemogenic program (Pereira et al., 2013).

In conclusion, this research finds that Gata2, Gfi1b, and cFos are sufficient for propagating hematopoiesis within MEFs. Etv6 in conjunction with these TFs increases reprogramming efficiency by roughly 2-6% as demonstrated by TF removal tests. The validity of this assessment can be tested against the presence of endothelial, blood, and stem cell markers in colonies transduced with the above stated TFs. These findings are of great value as they bring us a step closer to understanding the means by which we may treat hematological diseases through the establishment of an *in vitro* system capable of modeling hematopoietic disorders for distinction between normal and diseased hematopoiesis.

Because human and mouse hematopoiesis differ, our current work involves investigating the TFs and co-culture mediums that can be used to propagate an HSC program in *human fibroblasts*. Our work will follow the protocol and analyze the results detailed above to accomplish this task.

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