Engineering Notch Signaling in Stem Cells:
Towards Directed Generation of T Cells

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Statement of Purpose: Although a variety of micro-environmentally controlled events take place in the thymus, research has elucidated one of the main signaling pathways responsible for T-cell development: the notch-signaling pathway. The previously accepted in vitro system for studying T-cell differentiation was that of the fetal thymus organ culture system [1, 2]. *Although this system is capable of supporting T-cell development it is only able to produce a limited cellular yield.* Studies in T-cell differentiation have continued to progress by focusing on the usage of transfected stromal cells to present notch-ligands to embryonic and adult progenitor cells [3, 4]. HPCs cultured in the presence of DLL1 transfected cells gave rise to early T-cells indicating that the only extra cue necessary to create T-Cells is notch signaling [4]. Furthermore, we have previously shown that stromal cell contact is not needed to induce T-cell commitment and can be induced using notch ligand-coated microbeads [5]. ***Our design compensates for all of the limitations of the previously described culture conditions by presenting Notch ligands in a controllable manner on microbeads thus enabling the careful investigation of the spatial and temporal effects the ligands have on differentiation while removing the contaminating stromal cells.***

Methods: Here we purpose the usage of commercially available products: DLL4 (Axxora, San Diego, CA and R&D Systems, Minneapolis, MN) and protein coated microbeads (Spherotech, Lake Forest, IL and Bangs Laboratories, Fishes, IN), to present the DLL4 notch ligand. When the notch signaling pathway is activated via ligand binding, a series of intracellular events occur ultimately resulting in the release of the intracellular domain of the Notch receptor (ICN). The ICN will then translocate to the nucleus of the cell. Once inside the nucleus, the intracellular domain will associate with the CBF1/RBP-J transcription factor and activate Notch target genes. Using a dual-luciferase assay (Promega, Madison, WI or SABiosciences, Frederick, MD) we are able to quantify the amount of Notch signaling activated by measuring the amount of CBF1/RBP-J transcription taking place and then normalizing these results to a co-transfected control reporter gene.

Results: To assess the amount of notch signaling activated by DLL4 coated microbeads HEK293T cells were transfected with 4xwtCBF1Luciferase plasmid (generously provided by D. Hayward (Johns Hopkins School of Medicine, Baltimore, MD)), and pRL-TK reporter vector (Promega, Madison, WI) and subsequently incubated with microbeads coated with varying amounts of DLL4 ligand on the surface at different bead to cell ratios. Once measurements were taken, the ratio of luminescence from the CBF1/RBP-J transcription factor to the pRL-TK vector was calculated.

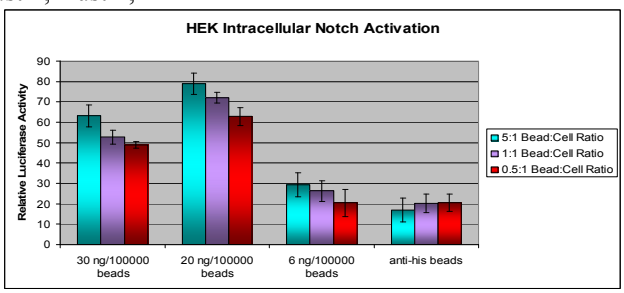

Figure 1: Notch signaling in HEK293T cells increases with increased DLL4 surface density as well as bead to cell ratio.

Investigations previously completed by our group have focused on microbeads that required a two step coating procedure. Investigations have begun on a new microbead system that would require only a single coating step, making DLL4 surface functionalization faster and more reliable. Characterization of the microbeads we purpose to use (Protein A coated microspheres (Spherotech, Lake Forest, IL)) shows that we can effectively control the number of Fc tagged molecules coated on each bead. This was done by incubation of the protein A coated beads with Fc-FITC molecules. The number of Fc molecules bound to each individual bead should correlate to the number of Fc-DLL4 molecules capable of binding to each microbead.

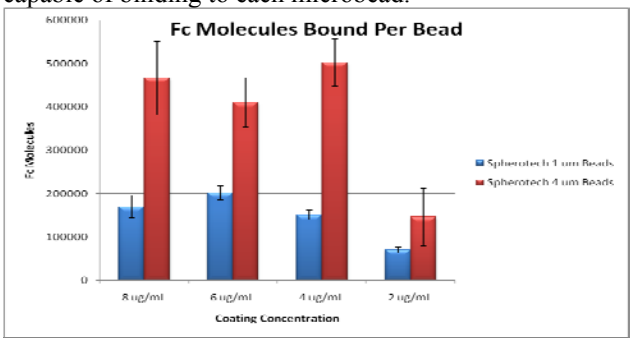

Figure 2: Spherotech Protein A coated microbeads can be functionalized with various concentrations of Fc tagged molecules

Conclusions: By focusing on this type of synthetic microbead system, it would be possible to systematically alter key variables within our culture system to create a highly uniform method to control stem cell differentiation and ultimately provide new insights into the cues necessary to induce sufficient notch to induce T cell differentiation. This data could ultimately be used in the development of a high-throughput system for the production of T cells for therapeutic applications.

Reference: [1] Res P, Blood. 1996:87:5196-5206
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