Notch Signaling Biomaterials: Efficient Intracellular Signaling and T cell Differentiation from Stem Cells

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Statement of Purpose: Recently, the role of Notch signaling in lymphocyte development, specifically T cell development, has been extensively characterized. Although Notch-ligand mediated signals have been shown to be a necessary component of T cell generation from stem cells, high-throughput, synthetic biomaterial-based systems for Notch-directed stem cell differentiation into lymphocytes are yet to be reported. We have recently reported a novel microbead-based, Notch signaling system to study stem cell differentiation into the T cell lineage [1]. Such a bead-based system provides effective Notch signaling to direct bone marrow-derived hematopoietic stem cells into the T cell lineage even in the absence of direct stem cell-stromal cell contact. We have further investigated the intracellular signaling effects of successful bead-cell binding in mouse embryonic stem cells. Our studies indicate that Notch functionalized microbeads demonstrate successful activation of Notch receptor. Such a bead-based artificial signaling system allows us to quantitatively study the effects of ligand density and signaling kinetics thereby providing further insights into the individual roles of Notch ligands in T cell differentiation and ultimately aid in the development of efficient technologies for the production of antigen-specific T cells for therapeutic applications.

Methods: Microbeads (Dynalbiotech, WI) were generated as described [1]. Bone marrow was isolated form C57/Bl6 mice (Jackson Laboratory, Maine) using standard protocols. Magnetically separated lin-c-kit+sca-1+ HPCs were seeded either on top of an OP9 cell (Tammy Reid, Canada) layer (mixed co-culture) or on cell culture filter inserts (Corning, VWR) (physically separated co-culture) along with SCF and IL-7 (Peprotech, NJ). Beads were added to HPCs (1:1) and uncoated beads were used as controls. On day 8, HPCs were stained for the B cell marker CD19 (Sigma, MO), and the early T cell marker Thy1.2 (eBiosciences CA) followed by FACS analysis. To demonstrate effective Notch signaling, stem cells were incubated with Notch functionalized beads and stained for activated Notch. Briefly, undifferentiated R1 embryonic stem cells (a gift from A. Nagy, Mount Sinai Hospital, Canada) were seeded (50000/coverslip) on poly-L-lysine coated coverslips one day prior to bead incubation. Uncoated and DLL4 functionalized microbeads were incubated with R1 cells for 1 hour prior to fixation and staining. Cells were stained with activated Notch1 antibody (Abcam, MA) and secondary FITC goat anti-rabbit antibody (Sigma-Aldrich, MO). Staining with DAPI (Invitrogen, CA) was used for nuclear visualization. Finally, coverslips were mounted and visualized using the Leica DM IRBE fluorescence microscope (Leica).

Results/Discussion: Figure 1A and 1B indicate that Thy 1.2^{+} early T cells were successfully generated from mouse bone marrow hematopoietic stem cells (BMHSCs) using DLL4 functionalized beads using both insert-based and mixed stromal cell (OP9) co-culture conditions, indicating that cell-cell contact is not necessary for DLL4 directed T cell differentiation [1]. Figure 1C and 1D indicate that these microbeads can successfully activate Notch signaling in stem cells. The intracellular portion of the Notch receptor is cleaved and translocates to the nucleus after successful receptor activation. Incubation of Notch functionalized beads with stem cells for 1 hour led to cleavage of Notch receptor and translocation to nucleus as demonstrated by localization of FITC and DAPI staining (Figure 1C). Stem cells without beads and with uncoated beads (data not shown) showed no trace of activated Notch receptor (Figure 1D).

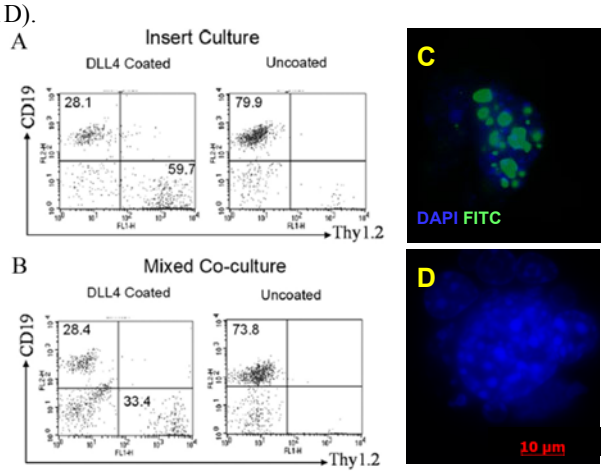

Figure 1. Notch ligand-coated microbeads can achieve Notch signaling in stem cells. Representative flow cytometry dot plots of Day 8 T-cell differentiation profiles in insert culture (A) and mixed coculture (B) from BMHSC with 1:1 bead-to-cell ratio. Embryonic stem cells were incubated with DLL4 coated beads at a 1:1 bead to cell ratio (C) and stained for activated Notch receptor (FITC) and nuclei (DAPI). Embryonic stem cells without beads served as negative controls (D).

Conclusions: Here, we have demonstrated that the Notch microbead system can effectively activate Notch1 receptor in stem cells and lead to efficient T cell development. Furthermore, studies are being conducted to explore the gene expression in Notch activated stem cells and quantitatively characterize the effect of varying bead to cell ratios.

References:

1. Taqvi, S., L. Dixit, and K. Roy, J Biomed Mater Res A. 2006 Dec 1;79(3):689-97.

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