Notch Signaling Biomaterials for the Generation of T cells from Hematopoietic Stem Cells

Sabia Taqvi[1], Lena Dixit and Krishnendu Roy[1].
[1]Department of Biomedical Engineering, University of Texas at Austin

Statement of Purpose:

With the advent of modern tissue-engineering concepts and emerging cellular transplantation therapies, stem cell-derived therapeutics are increasingly becoming a clinical reality. Currently, T cells for adoptive immunotherapy are obtained from a patient's peripheral blood. These methods are severely constrained by (a) the difficulties and inefficiency of patient cell isolation (b) problems with expansion of primary cells in vitro (c) the morbidity associated with autologous cell therapy and above all (d) the limited availability of donor cells. Therefore, technologies leading to efficient generation and expansion of therapeutic T cells from stem cells in a synthetic, controlled environment could provide a renewable, on-demand and readily available cell source for a variety of disease applications. Current systems for in vitro differentiation of T and B cells rely heavily on genetically transformed stromal cell lines in mixed cultures posing problems of cell separation and high-throughput differentiation. Notch signaling through stromal cell surface has been shown to play a critical role in T cell lineage commitment of hematopoietic and embryonic stem cells. We have developed an artificial stromal cell-based notch signaling approach (notch ligand functionalized magnetic microparticles) to direct embryonic or hematopoietic stem cells into T cells. The high throughput nature and easy to control cell signaling properties make the particle-based system an attractive option for ex vivo expansion and differentiation of stem cell-derived T cells.

Methods:

Microbeads (Dynalbiotech, WI) were functionalized with a HIS-tagged notch ligand (DLL4, R&D Systems, MN). OP9 (marrow stromal) cells were seeded in 24 well plates. Bone marrow was isolated form C57/Bl6 mice (Jackson Laboratory, Bar Harbor, Maine) using standard protocols. Lin-c-kit+sca-1+ HPCs were isolated using magnetic separation (Miltenyi Biotec, CA, and Dynalbiotech, WI). HPCs were seeded either on top of the OP9 cell layer (mixed co-culture) or on cell culture filter inserts (Corning, VWR) (physically separated co-culture) along with SCF and IL-7 (Peprotech, NJ) at 50ng/ml and 10ng/ml, respectively. Beads were added to HPCs (1:1) prior to seeding for 2 hours. Uncoated beads were used as controls. OP9 cells, beads, growth factors and medium were replaced every 4 days. On day 8, HPCs were stained for the B cell marker CD19 (R&D Systems), the early T cell marker Thy1.2 and respective isotype controls (eBiosciences, San Diego, CA) followed by FACS analysis.

Results / Discussion:

Figure 1 shows efficient T cell commitment of HPCs treated with notch ligand (DLL4)-functionalized microbeads as demonstrated by Thy1.2 (early T cell marker) expression. Control beads did not show presence of any T cells. These results indicate that in the presence of either direct or paracrine signaling from OP9 cells, notch-ligand functionalized magnetic microbeads (artificial thymic stromal cells) can direct HPCs into the T cell lineage. We are currently focusing on optimization of T cell differentiation and subsequent expansion of stem cell-derived T cells.

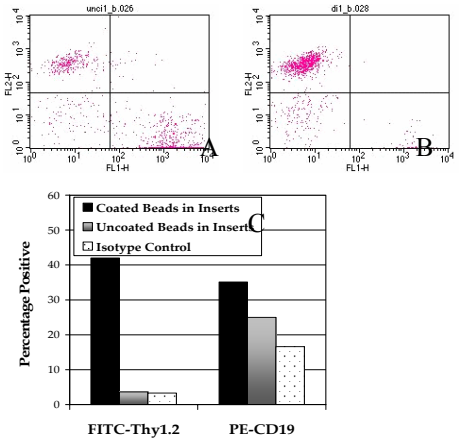

Figure 1: *Day 8 T cell differentiation in 2D insert culture from lin-cKit+sca-1+ BMHSC with 1:1 bead to cell ratio. (A) Notch ligand microbeads promote generation Thy1.2+ cells from hematopoietic stem cells. (B) In the presence of uncoated microbeads, hematopoietic stem cells differentiate to CD19+ cells. (C) Notch ligand-microbead results in efficient differentiation of T cells in insert culture. FL1=Thy1.2 FL2=CD19*

Conclusions:

Notch ligand functionalized microparticles represent an alternate, efficient method for differentiation of stem cells into hematopoietic and T cell lineages, respectively. The system serves as both a means of generating B and T cells ex vivo for immunodeficiency applications and a tool to better understand the cell-cell and cell-matrix interactions necessary in the areas of hematopoiesis and developmental biology. With such a quantitatively and temporally tunable bead based signaling coupled with paracrine signaling from OP9 stromal cells, T cell generation could be optimized and performed in a scalable, high through-put manner, making ex vivo T cell generation a practical approach in therapeutic applications.

References:

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