Clonally Selected Hematopoietic Stem Cells as a Tool for Gene Therapy

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Objective: Hematopoietic stem cells (SC) are promising targets for gene therapy but possibilities of unpredictable insertional oncogenesis possess major challenges to the clinical reality of gene therapy. *Ex vivo*, low proliferation of SC also limits the gene insertion into chromosome. In spite of all odds, theoretically, a successful SC gene therapy will require a single cell with a corrected gene at a safe zone of the chromosome. Considering above facts, our research was designed to investigate whether 1) single SC can be grown *in vitro* while keeping its self-renewal capacity and pluripotency and 2) cloned SC can protect lethally irradiated mice.

Methods: Freshly isolated mouse (C57BL/6) bone marrow SC (SCA+/c-kit+/Lin-) were sorted for single cell isolation on each well of a 96 well plate containing pre-grown culture of OP9 bone marrow stroma cells over-expressing notch ligand 1 (OP9-DL1). Separately, IL-7+Flt3L and M-CSF+IL-4+Flt3L were added to the culture media to support lymphopoiesis and myelopoiesis respectively. On 23rd day, cells were further expanded while clonality of each well was maintained. Cells from cloned SC were injected into lethally irradiated (950 CGy) syngeneic mice. All mice were observed for 180 days for bone marrow failure.

Results: Single sorted SC grown with IL-7 and Flt3L were differentiated into CD8+CD4+ and CD8+ T-cells whereas SC grown in the presence of IL-4, MCSF, and Flt3L were differentiated into myeloid cells. CD8+ cells were activated in MLR study. In spite of several sub-passages, 25% of clones were able to maintain SC markers (Sca1+C-Kit+Lin-). Additionally, cells (all lineage and stem cell marker containing cells) isolated from above clones were able to protect ~90% of syngeneic lethally irradiated mice. Ten percent of graft failure within the above group was observed between 27-36 days. Survived mice had all lineage cells similar to unirradiated normal mice. Stem cells transduced with a retrovirus carrying EGFP are currently being evaluated at clonal level with LAM-PCR to identify clonal vector proviral integration. Clones with correct genetic information will be used for syngeneic bone marrow transplantation (BMT).

Conclusion: While additional studies are required to further characterize the exact nature of the stem cells grown in the OP9-DL1 culture system, we conclude from the present data that self-renewing stem cells, mature T-cells, and myeloid cells can be produced and maintained at clonal level with this technique.

Translational Applicability: Because Fanconi anemia patients are more sensitive to the bone marrow transplant-conditional regimens such as high doses of cyclophosphamide and irradiation, an autologous *ex vivo* gene therapy may be considered as a better choice over allogeneic BMT. This investigation can be utilized to develop a relatively safer gene therapy for Fanconi anemia.