

Induction of CXCR4 in FANC-A, C, and D2^{-/-} Progenitor Cells to Enhance Hematopoietic Homing

A.M. Skinner¹, L. O'Neill¹, M. Grompe², P. Kurre¹

¹Oregon Health & Science University, Portland, OR; ²Oregon Stem Cell Center, Portland, OR

Bone marrow failure is a near-universal occurrence in patients with Fanconi anemia (FA) and thought to result from exhaustion of the hematopoietic stem cell (HSC) pool. Retrovirus mediated expression of the deficient FA gene corrects this phenotype and makes FA a candidate disease for gene therapy targeting autologous hematopoietic stem cells (HSC). However, limiting cell numbers, inherent cellular deficiencies in repopulation and compromised survival following transduction culture prevent therapeutically meaningful chimerism from genetically modified cells.

Objective: To offset numeric and functional disadvantages presented by FA HSC following *ex vivo* transduction culture, we sought to improve engraftment kinetics in murine models of FA. The chemokine receptor (CXCR) 4 is a principal factor in homing of hematopoietic progenitors to the bone marrow and is up-regulated by hypoxia inducible factor (HIF-1a). We hypothesized that hypoxia conditioning (<3.5% O₂) would up-regulate CXCR4 and concurrently limit the generation of reactive oxygen species (ROS) that contribute to *ex vivo* stem cell loss.

Methods: We examined baseline and induced levels of CXCR4 in Fanc^a, -c, and -d2 transgenic mice, as well as their wild type littermates following overnight incubation in hypoxic conditions. CXCR4 transcript was measured by quantitative real-time PCR and protein was quantified by flow-cytometry. ROS were detected with Amplex Red reagent (Invitrogen). Chimerism in transplantation experiments was evaluated in peripheral blood donor CD45.2 isotype (Fanc^c^{-/-} genotype) from murine recipients.

Results: We found that transgenic and wild type c-kit⁺ and sca-1⁺ progenitor cells express comparable baseline levels of CXCR4 transcript and protein, and exhibit similar up-regulation in response to hypoxia culture. Overnight incubation of Fanc^c progenitor cells in hypoxic conditions led to a 2-fold reduction in ROS compared to normoxia-conditioned cells. Transplantation of hypoxia- or normoxia-conditioned lineage-depleted Fanc^c^{-/-} progenitors set in a competitor assay with CD45.1 whole bone marrow cells into myeloablated murine recipients led to improved chimerism of hypoxia-conditioned Fanc^c^{-/-} progenitors in spleen but comparable chimerism in bone marrow and thymus when evaluated 5 days after transplant. When long-term chimerism was assessed in blood at 16 weeks, hypoxia-conditioned progenitors exhibited improved chimerism compared to normoxia-conditioned counterparts.

Conclusions: These studies provide evidence for the first time that CXCR4 regulation in mice representing multiple FA genotypes is intact and that modulation of CXCR4 protein expression may offer a viable strategy to improve homing in a model with established repopulation deficiency.

Translational Applicability: Given problems with *ex vivo* modification of HSC, the novel strategy proposed herein may help overcome some of those limitations.