



48th ASH Annual Meeting

Orange County Convention Center, Orlando, Florida

December 9-12, 2006

Ferritin Heavy Chain Stimulates HbS-to-HbF Switching in Erythroid Precursor Cells from Sickle Cell Patients

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We have found that ferritin heavy chain (FtH), an antioxidant/stress response/iron-storage protein, localizes to the nucleus in K562 cells and represses the human adult beta-globin promoter in transient assays in primate cells (Broyles et al., *PNAS* 98: 9145, 2001). Since other work indicates FtH is also a gene activator of fetal-globin genes, we hypothesize that FtH is a long-sought developmental hemoglobin (Hb) switching factor and that delivery of FtH to human adult erythroid cell precursors will reverse the phenotype to HbF, offering a phenotypic cure for sickle cell disease (SCD). Chromatin immunoprecipitation (ChIP) assays, antisense treatments, and an FtH transgenic mouse have confirmed that FtH is a globin gene regulatory protein *in vivo*. With erythroid precursor cells from pediatric SCD patients, under an IRB-approved protocol, we have used a two-phase culture system for *in vitro* maturation of erythroid cells in the presence of FtH, delivered to the cells as pure protein, as an expression plasmid, or as a priority inducer compound that activates the endogenous FtH gene. HPLC with a PolyCAT A column was used to separate and quantify human Hbs. With each mode of delivery, FtH stimulated a complete switch from HbS to HbF. This result was repeatable in multiple experiments using erythroid precursor cells from three different SCD donors. Fluorescently-labeled recombinant human FtH protein was taken into red cell precursors in culture, suggesting that the purified protein can be directly delivered without gene therapy. This method of producing a phenotypic cure in SCD patients should be easy and inexpensive to deliver *in vivo*.

Certification for Human Subjects: I certify that this study abides by the rules of the appropriate internal review board and the tenets of the Helsinki protocol