



GENE REGULATORY PROTEIN THERAPY USING FERRITIN HEAVY CHAIN: TOWARDS A PHENOTYPIC CURE FOR SICKLE CELL DISEASE

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Ferritin heavy chain (FtH), an embryonically-expressed protein in the erythroid lineage, localizes to the nucleus and represses the human adult β -globin promoter in transient assays (Broyles et al., *PNAS* 98: 9145, 2001). Previous work indicates that FtH is also a gene activator of γ -globin (Wu & Noguchi, *JBC* 266: 17566, 1991). We have hypothesized that FtH is a key, long-sought developmental hemoglobin (Hb) switching factor and that appropriate delivery of FtH to human erythroid cell precursors may partially reverse the phenotype to HbF, offering a phenotypic cure for sickle cell disease.

ChIP (chromatin immunoprecipitation) assays with cross-linked chromatin of K562 cells in which the β -globin gene is repressed, using anti-FtH polyclonal antisera, strongly indicate that FtH occupies the repression site (a CAGTGC motif) *in vivo*. Binding to this -150 site is required for β -promoter repression in co-transfections. An Alexa488-tagged antisense oligonucleotide to FtH transfected into K562 cells enters the nucleus and derepresses the β -globin gene while deactivating γ -globin expression. It is not clear whether iron is required for the DNA sequence-specific binding of FtH. Although FtL and the 222 FtH mutant (which does not bind iron) do not bind to the β -globin promoter site, FtX which has an amino acid substitution in the ferroxidase center does show binding activity *in vitro*.

The mouse β Major-globin promoter has an analogous CAGTGN motif at -160 bp from the cap site that competes specifically with the human CAGTGC site for K562 nuclear FtH binding. When the mouse sequence is used as the probe, three bands are obtained with K562 nuclear FtH; two of the bands are competed by the human CAGTGC promoter sequence, and the other band is competed by an authentic GATA-1 oligo, suggesting a possible interaction between GATA-1 and FtH. The mouse β Minor-globin promoter lacks the -150/-160 CAGTGN motif and, therefore, the FtH binding site. Thus, a human FtH transgenic mouse, in which the hFtH gene is driven by a truncated β -promoter lacking the CAGTGN motif, should express hFtH in definitive erythroid cells where the hFtH would be predicted to repress β Major-globin but not β Minor-globin, resulting in a decreased β Major/ β Minor ratio in the definitive erythroid cells and a mild β -thalassemia due to excess α chains. Preliminary results from litters of founder hFtH-tg mice indicate that such is the case, i.e., that human FtH functions as a β Major-globin repressor *in vivo*, creating a mild β -thalassemia. In humans, excess α -chains would not be expected due to a compensatory increase in γ -globin expression (Dover & Boyer, *Blood* 69: 1109, 1987).

Experiments are underway to test the ability of FtH to repress β S-globin expression and increase γ -globin expression in erythroid precursor cells from blood of pediatric sickle cell patients undergoing transfusion therapy, under an IRB-approved protocol, as well as in erythroid precursors from normal donors.