



International BioIron Society

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

*Robert H. Broyles*¹, *Robert A. Floyd*¹, *Visar Belegu*¹, *Austin C. Roth*¹, *Charles Stewart*¹, *Quentin Pye*¹, *Biji Kurien*¹, *Klodiana Jani*², and *Marie Trudel*². ¹Oklahoma Medical Research Foundation, Oklahoma City, OK 73104 USA, and ²Institut de Recherches Cliniques de Montréal, Montréal, Quebec H2W 1R7 Canada.

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 expression assays (Broyles et al., *PNAS* **98**: 9145, 2001). Recently, we have performed chromatin immunoprecipitation (ChIP) assays with cross-linked chromatin of K562 cells in which the β -globin gene is repressed, using anti-FtH polyclonal antisera. These results 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. It is not clear whether iron is required for this sequence-specific binding. Although FtL and the 222 FtH mutant (which does not bind iron) do not bind to the β -globin promoter site, a recently discovered ferritin (FtX) which has an amino acid substitution in the ferroxidase center does show binding activity *in vitro*. Competitive EMSA assays have revealed that 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 FtH gene is driven by a truncated β -promoter lacking the CAGTGN motif, should express human FtH in definitive erythroid cells where the FtH would be predicted to repress β^{Major} -globin but not β^{Minor} -globin. Such a mouse would be predicted to survive but be born with a mild β -thalassemia due to the decreased $\beta^{\text{Major}}/\beta^{\text{Minor}}$ ratio in its definitive erythroid cells. Preliminary results from litters of founder FtH-tg mice indicate that such is the case, i.e., that human FtH functions as a β^{Major} -globin repressor *in vivo*. FtH-tg mice with high copy numbers of the transgene have greater numbers of target cells, due to inclusions of excess α -globin. In applying this therapy to humans, thalassemia would not be expected because FtH has also been reported to activate γ (fetal)-globin gene expression in co-transfection experiments. Our aim is to repress mutant β -globin expression and increase γ -globin expression in human erythroid precursor cells using a recently discovered priority compound that is a powerful inducer of endogenous FtH in human cells in culture.

26 May 2005

Oral presentation, IBIS, Prague, The Czech Republic, May, 2005.